Vol. XLIII No. 8

August 1954

SCIENTIFIC EDITION

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

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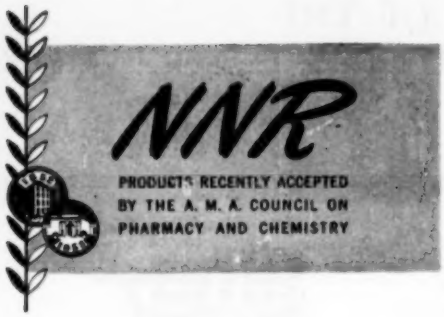
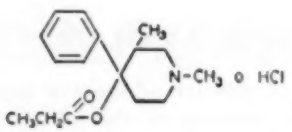


ALPHAPRODINE HYDROCHLORIDE.—Nisentil Hydrochloride (Hoffmann-LaRoche).—$C_{18}H_{23}NO_2 \cdot HCl$.—M. W. 297.82.—1,3-Dimethyl-4-phenyl-4-piperidyl propionate hydrochloride.—The structural formula of alphaprodine hydrochloride may be represented as follows:



Actions and Uses.—Alphaprodine hydrochloride is a short-acting synthetic, narcotic analgesic agent, chemically resembling meperidine, but unrelated chemically to morphine. The analgesic action of alphaprodine, like that of morphine, is associated with euphoria, mild sedation, slight dizziness, itching, and diaphoresis, but is accompanied by less nausea, vomiting, or respiratory depression. Its analgesic and depressant actions are somewhat less intense, but more prompt and of shorter duration, than those of morphine. The relatively short duration of analgesic and sedative effect minimizes the hazard of respiratory depression resulting from the drug; but, if barbiturates are used concomitantly, the tendency to depressed respiration in the newborn may be increased because alphaprodine hydrochloride passes freely across the placental barrier. Its rapid onset and relatively short action permit considerable flexibility of administration. It is suited primarily for temporary analgesia in obstetrics, for urologic examinations and procedures (particularly cystoscopy), preoperatively in surgery, and for minor surgical procedures, especially in orthopedics, ophthalmology, rhinology, and laryngology. The drug may be used in conjunction with nerve block or inhalation anesthesia and with barbiturate sedation when allowance is made for the added depressant effect.

Alphaprodine hydrochloride produces little or no cumulative effect, but tolerance that involves the liability of addiction can develop. For this reason, the drug is subject to restriction as a narcotic. Although it is intended only for temporary analgesia, the potential addiction liability of its prolonged use for other purposes or by addicts as a substitute for other narcotic analgesics should be kept in mind.

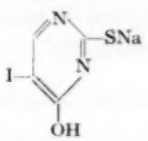
Dosage.—Alphaprodine hydrochloride is administered in solution by subcutaneous injection; intravenous injection may be employed when very rapid and brief analgesia is desired. The average initial subcutaneous dose is 40 to 60 mg., depending on the patient's weight; similar doses may be repeated at two-hour intervals. A dose of 40 mg. is suggested for a patient weighing 50 Kg. (110 lb.). Such a dose usually produces analgesia within five minutes and lasts for an average of two hours. In obstetrics, the initial dose may be given at any time after the cervix has begun to dilate. Depression of fetal respiration resulting from the drug is largely obviated if the last subcutaneous dose is administered at least two hours prior to delivery. The average intravenous dose ranges from 20 to 30 mg., usually produces analgesia within one to two minutes, and lasts thirty to sixty minutes. In the event of overdosage, nalorphine hydrochloride is an effective antidote.

Tests and Standards.—

Physical Properties: Alphaprodine hydrochloride is a white, crystalline, bitter powder with an amine-like odor, m. p. 218–221°. It is freely soluble in alcohol, in chloroform, and in water and very slightly soluble in ether. Alphaprodine hydrochloride is stable to air, light, and heat. The *p*H of a 1% solution is 4.5–5.2.

[For more information regarding this monograph, see *J. Am. Med Assoc.*, **155**, 1159(July 24, 1954).]

IOTHIOURACIL SODIUM.—Itrumil Sodium (Ciba).—$C_4H_2IN_2NaOS$.—M. W. 276.06.—Sodium 5-iodo-2-thiouracil.—The structural formula of iothiouracil sodium may be represented as follows:



Actions and Uses.—Iothiouracil sodium, an organic chemical iodine derivative of thiouracil, exhibits the thyroid-involuting effect of iodine and the antithyroid action (inhibition of thyroxin or thyroglobulin) of the parent drug. Iothiouracil sodium induces less thyroid vascularization and goitrogenic effects (increased thyroid hyperplasia, gland size, and friability) than noniodinated thiouracil compounds. Although animal experiments indicate that iothiouracil sodium is more readily taken up by the thyroid than noniodinated derivatives, clinical evidence so far obtained does not warrant the conclusion that the drug is superior to noniodinated derivatives administered concomitantly with iodine. Iothiouracil sodium is broken down in the body into its thiouracil and iodine portions, which are excreted separately.

Iothiouracil sodium is indicated in the preoperative management of hyperthyroidism, in the treatment of patients for whom thyroidectomy is contraindicated, and in the treatment of postoperative recurrent hyperthyroidism. It should be used with caution during pregnancy and should be discontinued during the last few weeks of pregnancy to prevent complications in the newborn; infants should not be nursed by mothers receiving therapy. It should be employed cautiously in persons known to be sensitive to iodine.

Like other thiouracil derivatives, iothiouracil sodium is associated with a lower incidence of toxic effects than the parent compound, thiouracil, but likewise may produce serious reactions that require cessation of medication. These include drug fever, skin rash, severe leukopenia, granulocytopenia, and swelling of the cervical lymph nodes. A leukocyte and differential blood count should be made before treatment because of the frequent "spontaneous" appearance of leukopenia in hyperthyroidism; regular blood counts should be made during therapy and the patient instructed to report the appearance of any adverse symptoms.

Dosage.—Iothiouracil sodium is administered orally. In any given dose, iodine accounts for approximately 50%, the thiouracil molecule for 40%, and the sodium ion for 10% of the prescribed amount.

For preoperative management, an initial daily dosage of 150 to 200 mg. (divided into doses of 50 mg. three or four times daily) may produce a satisfactory response in many patients, but most thyrotoxic patients require a daily dosage of 300 mg. (100 mg. three times daily) to produce rapid and complete remission. The established effective dosage for each patient should be continued until the disease has been controlled satisfactorily. For optimal preoperative results, from the standpoint of decreased vascularity and friability of the gland, at least four weeks of therapy are recommended; severely ill patients may not respond adequately until after eight weeks. If a patient does not improve after a month of treatment at the minimum daily dosage level of 150 mg., the dosage should be increased to 300 mg. daily. If a second month of therapy at the higher dosage level fails to produce satisfactory response, the drug should be discontinued. Rarely, a daily dosage level of 600 to 800 mg. may be instituted to control refractory patients.

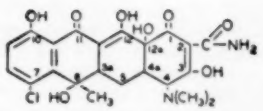
For the treatment of patients in whom thyroidectomy is contraindicated, or in instances of postoperative recurrence, the initially effective dosage may be gradually reduced to an adequate maintenance level. Therapy may be discontinued and resumed as required to keep the disease in check or to avoid untoward toxic effects; some patients cannot be kept in remission with continuous therapy.

Tests and Standards.—

Physical Properties: Iothiouracil sodium is an odorless, white to light yellow, crystalline powder, m. p. 235–240° (with decomposition). The approximate amounts that dissolve at 25° in the following solvents to form 100 ml. of solution are: 0.5 Gm. in alcohol and 3.5 Gm. in water. It is practically insoluble in acids. Iothiouracil sodium is usually obtained as the dihydrate which is reasonably stable to moisture and sunlight at room temperature. The *p*H of a 2% solution is 8.5–9.5.

[For more information regarding this monograph, see *J. Am. Med. Assoc.*, **155**, 444(May 29, 1954).]

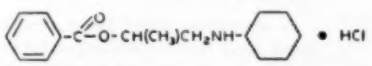
AUREOMYCIN CALCIUM.—The calcium salt of 7-chloro-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro - 3,6,10,12,12a - pentahydroxy - 6 - methyl - 1, - 11-dioxo-2-naphthacenecarboxamide. It complies with the requirements of the Federal Food and Drug Administration. The exact chemical formula of aureomycin calcium is unknown. It is believed that two of three acid hydrogens of aureomycin are replaced by calcium. The structural formula of aureomycin may be represented as follows:



Actions and Uses.—Aureomycin calcium has the same actions and uses as the hydrochloride salt. See New and Nonofficial Remedies under the monograph on aureomycin hydrochloride. The calcium salt is useful for oral administration of the antibiotic in concentrated form for patients who cannot take dry forms of the drug.

Dosage.—Aureomycin calcium is administered orally in the form of drops for infants and in the form of syrup for children or adults who do not readily take dry forms of the drug. The dosage for the calcium salt is expressed in terms of the hydrochloride salt.

HEXYLCAINE HYDROCHLORIDE.—Cyclaine Hydrochloride (Sharp & Dohme).—$C_{16}H_{23}NO_2.HCl$. —M.W. 297.82.—1-Cyclohexylamino-2-propyl benzoate hydrochloride.—The structural formula of hexylcaine hydrochloride may be represented as follows:



Actions and Uses.—Hexylcaine hydrochloride is a soluble local anesthetic suitable for infiltration, spinal (subarachnoid) anesthesia, surface (topical) anesthesia, and nerve block, including caudal (extradural sacral) block and lumbar epidural block. Animal experiments indicate that the onset of its anesthetic effect is rapid and that, from the standpoint of duration of anesthesia and degree of motor paralysis produced, it compares favorably with equal concentrations of the more active local anesthetic compounds in use. When applied topically, it is at least as potent as equal concentrations of cocaine. Clinical studies also indicate that, when used for infiltration and nerve block, it is faster and longer acting than an equal concentration of procaine.

Hexylcaine hydrochloride shares the toxic potentialities of other local anesthetic agents and should be employed with the same precautions and in accordance with established techniques of administration.

Dosage.—Hexylcaine hydrochloride should be administered in the smallest dose that will give the required anesthesia.

For infiltration anesthesia to relieve local pain, 5 to 65 cc. of a 1% solution is injected into the affected areas; up to 90 cc. of the same concentration may be required for surgical procedures. More dilute solutions may be used with a corresponding increase in the volume to be injected. For most nerve blocks, 2 to 10 cc. of a 1% solution is recommended. For single-injection caudal (extradural sacral) block, the dose is 25 to 35 cc. of a 1% solution; for continuous caudal block, 20 to 50 cc.; and for lumbar epidural block, 25 to 50 cc.

(Continued on page V)

1955 Iodine Research Award Nominations Requested Prior to January 1, 1955

Nominations are now being received by the AMERICAN PHARMACEUTICAL ASSOCIATION for the 1955 Chilean Iodine Educational Bureau, Inc. Award recognizing outstanding research in the chemistry and pharmacy of iodine and its compounds as applied in pharmacy or medicine.

The award consists of $1,000 and a diploma setting forth the reasons for selection of the recipient. It may be presented each year at the annual meeting of the ASSOCIATION.

Any member of the ASSOCIATION may propose a nominee by submitting eight copies of each of the publications to be considered in the competition, a biographical sketch of the nominee including date of birth, and a list of his publications. Eight copies of the nomination must be submitted to Robert P. Fischelis, Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION, 2215 Constitution Ave., N. W., Washington 7, D. C. To be eligible for the 1955 Award, *nominations must be received on or before January 1, 1955.*

A nominee must be a resident of the United States or Canada. He must have accomplished outstanding research in the chemistry or pharmacy of iodine and its compounds as applied in pharmacy or medicine.

During the period covered by the nomination the nominee shall have been actively engaged in, shall have completed, or shall have published a report upon the line of investigation for which the award is made. During the period of two years prior to the date of nomination, the nominee shall not have been engaged in research under the sponsorship of the Chilean Iodine Educational Bureau, Inc.

The recipient will be selected by an award committee which is appointed by the chairman of the ASSOCIATION'S Council. The present committee includes Justin L. Powers, chairman; Paul Block, E. A. Brecht, Leroy D. Edwards, Harvey B. Haag, Lloyd M. Parks, and Heber W. Youngken.

The recipient will deliver a paper or lecture upon the subject of his scientific work at the meeting at which the award is conferred. His paper, or address, will then be published in the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION. Travel expense to the meeting at which the award is to be made is provided for up to $250.

The award is now in its seventh year. The recipient of the first award was Dr. William T. Salter, Yale University pharmacologist; the second recipient was Dr. George M. Curtis, Chairman of the Department of Surgical Research and Professor of Surgery at Ohio State University, and the third was Dr. C. P. Leblond, Professor of Anatomy, McGill University, Montreal, Canada. The fourth award was shared jointly by Dr. George Moore of the University of Minnesota Medical School and Dr. Moses Ashkenazy, Chief of Neurosurgery, Veterans Hospital, Houston, Tex. Dr. Vernon H. Wallingford, Mallinckrodt Chemical Works, St. Louis, Missouri, was the recipient of the fifth award. Dr. Jack Gross, Associate Professor of Anatomy, State University of New York, College of Medicine in Brooklyn received the sixth award which was conferred during the 1954 Convention of the AMERICAN PHARMACEUTICAL ASSOCIATION.

N.N.R.

• • • • • • • • • • • • • • *from page III*

For single-dose spinal anesthesia the amount to be injected is 15 to 50 mg. administered by diluting 0.6 to 2 cc. of a 2.5% solution containing 10% dextrose with 0.3 to 2 cc. of aspirated spinal fluid, depending on the final hyperbaric concentration of dextrose desired, the site and type of surgical operation, and the condition of the patient. A total volume (after appropriate dilution) of 4 cc. is usually employed for operations above the level of the umbilicus and a total of 1 to 3 cc. for surgery below that level. The position of the patient is altered to achieve the exact level of anesthesia desired. For low spinal anesthesia of obstetric patients during delivery, a dose of 20 to 25 mg. (0.8 to 1 cc. of the 2.5% solution) is injected without barbotage at the third or fourth lumbar interspace and the position of the patient regulated to obtain anesthesia extending to a level of 5 to 9 cm. below the umbilicus. For cesarean section, 25 mg. is usually employed and the patient positioned to obtain anesthesia to the level of the umbilicus. For fractional spinal anesthesia, an initial dose of 15 to 30 mg. is recommended, followed by 15 mg. every 20 to 30 minutes after the first 30 minutes, administered by diluting 6 cc. of the 2.5% solution to 10 cc. with spinal fluid to make a 1.5% concentration (15 mg. per cubic centimeter).

For surface anesthesia, a maximum concentration of 5% may be applied topically by means of cotton applicators, packs, spray, or gargle, depending on the site to be reached A minimum concentration of 1% may be sufficient for certain minor procedures. For endotracheal intubation, 2 to 10 cc. of a 5% solution may be sprayed on the pharynx and larynx. Gargling with about 5 cc. of the 5% solution may be used prior to bronchoscopy and followed by placement of saturated cotton applicators in the pyriform spaces. For surface anesthesia of the urethral mucous membrane, 5 to 10 cc. of the 5% solution is used by instillation and retention for 5 to 10 minutes in the male urethra; a cotton applicator saturated with the 5% solution is inserted into the female urethra for 10 minutes.

Tests and Standards.—

Physical Properties: Hexylcaine hydrochloride is a white, bitter powder with a slight aromatic odor, m. p. 182–184°. It is freely soluble in alcohol and in chloroform and practically insoluble in ether. The approximate amount that dissolves at 25° in water to form 100 ml. of solution is 6 Gm. The *p*H of a 5% solution is 4.1–4.7.

[For more information regarding this monograph, see *J. Am. Med. Assoc.*, **155,** 908(July 3, 1954).]

W. H. O. Proposes New Non-Proprietary Name

The following name is now under consideration by the World Health Organization as a proposed non-proprietary name:

PROPOSED INTERNATIONAL NON-PROPRIETARY NAME (Latin, English, French)	CHEMICAL NAME OR DESCRIPTION (English, French)
levallorphanum levallorphan levallorphane	(−) 3-hydroxy-*N*-allylmorphinan (−) hydroxy-3-*N*-allylmorphinane

The above name, in accordance with paragraph 3 of the Procedure of the Selection of Recommended International Non-Proprietary Names for Drugs moving in International Commerce, was published in the *Chronicle of the World Health Organization* **8,** 216(June 1954). Comments on, or formal objections to, the above name may be filed within a period of six months from July 1, 1954, and should be forwarded to the Director-General, World Health Organization, Palais des Nations, Geneva, Switzerland.

A formal objection to a proposed name must include the identity of the person objecting, a statement of his interest in the name, and a statement setting forth the reasons for his objection to the name proposed.

In accordance with the procedure agreed upon by the World Health Organization [*Chronicle of the World Health Organization,* **7,** 269–324(1953); *Drug Standards* **22,** 16–23(1954)], the Public Health Service has released the foregoing information to the AMERICAN PHARMACEUTICAL ASSOCIATION and other authorized agencies for dissemination. This is in line with previous cooperative arrangements which make it possible for organizations concerned with the introduction and protection of non-proprietary names for therapeutic substances to comment upon the proposed non-proprietary names and to determine whether proposed names conflict with proprietary rights existing in the United States.

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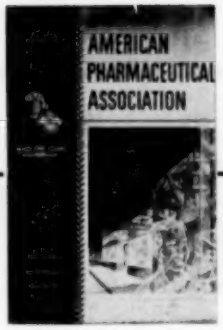

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In the August 1954 issue of the Practical Pharmacy Edition

(All references to actions and uses are taken from the statements of the producers of the products mentioned.)

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▸ **The American Druggists' Fire Insurance Co.** re-emphasizes that it is "The Druggists' Own Company."

▸ **Clinitest** *Sealed-in-Foil Reagent* tablets are offered by **Ames** as a "practical, profitable suggestion."

▸ Dependability is the attribute stressed by **Chesebrough** in describing its **Vaseline** sterile petrolatum gauze dressings.

▸ **Lactinex** tablets are advocated by **Hynson, Westcott & Dunning** for the effective treatment of diarrhea.

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▸ **Merck & Co.** takes justifiable pride in the fact that its name "stands for purity, uniformity, and dependability."

▸ **Benadryl,** "for relief in a wide variety of seasonal allergies" is available through **Parke, Davis & Company.**

▸ Qualified students can matriculate at **Philadelphia College of Pharmacy and Science** for courses leading to B.Sc., M.Sc. and D.Sc. degrees. **P.C.P. & S.** also has schools of Chemistry, Bacteriology and Biology.

▸ Attention is focused by **Roerig** on its new **Tetracyn Ophthalmic Ointment.**

▸ **Penalev** is suggested by **Sharp & Dohme** "for recurrent rheumatic fever prophylaxis."

▸ **Smith, Kline & French** has reserved the first page to announce **Vasocort** for allergic rhinitis and hay fever, and in an additional insertion, stresses the sales potential of **Benzedrex Inhalers.**

▸ The proven effectiveness of **Kaopectate** for the treatment of diarrhea is reaffirmed in **The Upjohn Company's** insertion.

▸ New from the laboratories of **U. S. Vitamin Corporation** is **Geriatrone Elixir,** "a delightfully flavored digestive-nutritive tonic."

▸ For the treatment of seasonal allergies, **Multihist's** "full therapeutic action with virtual freedom from side effects" is spotlighted by **Smith-Dorsey.**

▸ **"Peritrate** is the biggest selling drug for the prevention of attacks in angina pectoris," says **Warner-Chilcott.**

▸ **Winthrop-Stearns** reports excellent results from the use of **Alevaire** in liquefying pulmonary secretions.

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Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

VOLUME XLIII — *AUGUST, 1954* — NUMBER 8 — CONSECUTIVE No. 16

Repeated Intravenous Infusions of Oxypolygelatin (OPG) Solution in Irradiated Rabbits*

By LAWRENCE E. DETRICK, VIRGINIA DEBLEY, and THOMAS J. HALEY

Seven-hundred r whole irradiation increased the mortality rate of rabbits given eleven consecutive daily intravenous infusions of a modified oxypolygelatin solution. Irradiated but noninfused control animals all survived the thirty-day experiment. Early deaths in the irradiated infused animals, 25 per cent within one and one-half to five hours after injections were started, were attributed to cardiac failure. Later deaths, 62 per cent occurring one to eleven days after X-irradiation, were accompanied by hypervolemia, edema, hemorrhage, infection, and pathological changes in the liver, lung, and kidney.

WHOLE BLOOD, plasma, and blood derivatives are considered more desirable than "blood substitutes" for intravenous administration but in case of an atomic emergency, supplies of blood products would soon be exhausted (1–3). Whole blood transfusions were of only temporary benefit to Japanese bomb patients (4) and of possible benefit to Bikini goats (5). When blood transfusions were administered to irradiated dogs no beneficial effect was observed on the mortality rate (6), they may have been deleterious to survival (7), or their use may have been a hazard (8). Even though several "blood substitutes" have been approved for stockpiling and use in case of an atomic emergency, there are no clinical or experimental data to support their employment in postirradiation therapy.

The present experiment was undertaken to confirm an observation previously reported that irradiation increased the sensitivity to and the mortality rate of rabbits receiving repeated intravenous infusions of tapioca dextrin (9). A clinically accepted particle size modified OPG solution, which does not cause initial fatalities in normal animals (10), has been substituted for the small molecular-weight tapioca dextrin solution used previously and found to decrease survival of irradiated animals (9).

EXPERIMENTAL

Twenty-five female Dutch rabbits (2,035 Gm. average body weight) were given 700 r acute whole body irradiation. Nine irradiated noninfused animals served as controls (Fig. 1, *A*). Sixteen irradiated animals (Fig. 1, *B* and *C*) were given 11 consecutive daily intravenous injections (150 ml. daily) of OPG solution containing amino acid hydrolysate and B complex vitamins (10) in two three-hour infusion periods with an hour rest period intervening. Injections were made into the jugular veins via an indwelling polyethylene catheter and were started one hour postirradiation using the method reported previously (11). Radiation was administered from above and below with two 250 KVP

* Received November 16, 1953, from the Division of Pharmacology and Toxicology, Atomic Energy Project, School of Medicine, University of California, Los Angeles.

Presented at the fall meeting of The American Society for Pharmacology and Experimental Therapeutics, September 7–9, 1953, New Haven, Conn.

This paper is based on work performed under contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.

The authors wish to thank Dr. Alvin E. Lewis and Mrs. Dorothy N. Highby for the preparation and interpretation of the histological material and to express their appreciation to Don Baxter, Inc., Glendale, Calif., for their cooperation in the preparation of the parenteral solutions.

Picker Industrial Units operating simultaneously. The technical factors were: 15 Ma; SFD 50 cm.; filters Cu 0.21 mm. inherent and 0.5 mm. parabolic, 1 mm. Al; HVL 2.0 mm. Cu; rate 74.4 r/minute measured in air with a Victoreen Thimble r-meter.

RESULTS

The effect of irradiation on the group average body weight changes of the orally fed animals that had an uneventful recovery is shown in Fig. 1, *A*. Individual body weight changes and the death distribution frequency of irradiated-OPG-infused animals are shown in Fig. 1, curves *B* and *C*, the separation of which was for visual clarity only. Twenty-five per cent of this group that died from one and one-half to five hours after infusions began were omitted from curves *B* and *C* but are considered in Tables I and II together with the distribution of deaths that occurred during the experiment. Both initial and later deaths were precipitous in nature and occurred soon after the onset of convulsions.

When sacrificed and autopsied thirty days post-irradiation, the organs of the orally fed group (Fig. 1, *A*) appeared essentially normal on gross examination. Table I shows the comparative necropsy findings of the irradiated-OPG-infused animals that died (Fig. 1, *B* and *C*), including the early deaths. Initial deaths (one to five hours) were accompanied by an engorgement of the heart and adjacent vessels, an empty appearance of the large vessels over the stomach and somewhat distended intestinal vessels. Animals that died one to three days postirradiation showed the beginnings of liver, lung, and kidney damage (Table I). These gross changes became intensified in deaths occurring seven to thirteen days postirradiation and in addition included engorgement of the heart, coronary and adjacent vessels; hemorrhage in the lung, heart, and gastrointestinal tract; tarry intestinal content, watery stools; and kidneys of a translucent amber appearance. These findings were somewhat decreased in a single animal that died seven days after infusions were discontinued.

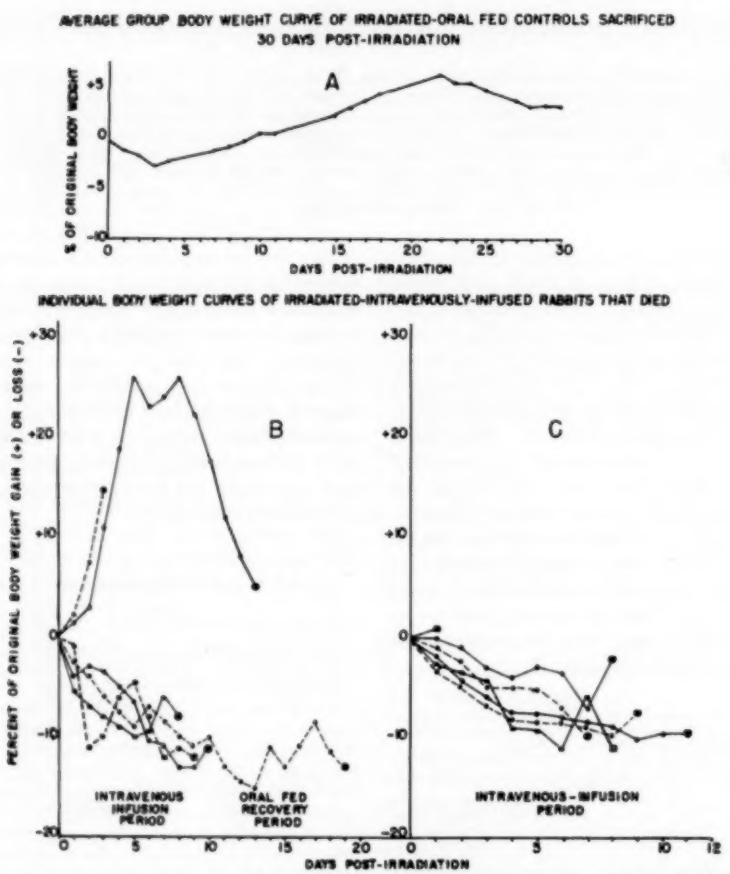

Fig. 1.—Individual body weight curves of irradiated-intravenously infused rabbits and controls.

TABLE I.—GROSS NECROPSY FINDINGS IN IRRADIATED-OPG-INFUSED RABBITS

		Time of Death			
		Days		Days	
	Hrs., 1½–5[a]	1–3	7–11	13[b]	19[b]
No. of animals	4	2	8	1	1
Edema:					
Ascitic	0	0	+	+	+
Pleural	0	+	+	+	0
Pericardial	0	0	+	0	0
Liver	Normal	Pale, mottled, granular cut surface	Intensified over preceding group	Diminished in intensity	Less mottled
Kidney	Normal	Large, soft, pale	Large, soft, translucent amber	Improved over preceding group	Brown with pink cast
Lungs	Normal	Pale, mottled, focal congestion	Petechiae, hemorrhage, consolidated areas	Diminished from preceding group	Pale, otherwise normal
Heart	Dilated, engorged great vessels	Great vessels congested	Dilated heart, engorged coronaries, petechiae, hemorrage in pericardium	Engorged, few petechiae	Normal
GI Tract	Stomach veins empty. Small intestinal veins filled. Pink fluid in intestines.	*Idem*	Vessels engorged, petechiae, hemorrhage, content tarry	*Idem*	Normal

[a] Early deaths not shown, Fig. 1, curves *B* and *C*.
[b] Infusions discontinued after 11 consecutive daily injections.

TABLE II.—HISTOLOGICAL CHANGES IN IRRADIATED-OPG-INFUSED RABBITS

		Time of Death			
		Days		Days	
	Hrs., 1½–5[a]	1–3	7–11	13[b]	19[b]
No. of animals and % mortality	4 (25%)	2 (12%)	8 (50%)	1 (6%)	1 (6%)
Liver glycogen	Normal	Decreased	None	None	Increased, but not normal content
Liver	Lymphocytic infiltration of hepatic triads	Congestion, hepatitis, vacuolization of hepatic nuclei	Intensified over preceding group	*Idem*	Diminished intensity
Kidney, hydropic degeneration[c]	Normal	0–2+	4+	4+	2+
Other changes	Focal areas interstitial nephritis	Swollen convoluted tubules	Tubular degeneration, nuclei normal, lumen narrowed	Scattered extramedullary hematopoiesis	Improved but not normal
Lungs	Lymphocytic degeneration due to X-irradiation, congestion	Congestion, pneumonia diffuse	Chronic passive congestion, pneumonia diffuse, alveolar collapse, acute hemorrhage, small infarcts	Congestion, hemorrhage, pneumonia	Essentially normal
Spleen[d]	Congestion, lymphocytic	Reticuloendothelial, hyperplasia in follicles	Congestion, dissolution of follicle	Diminished, intensity	Improved, but not normal

[a] Early deaths not shown, Fig. 1, curves *B* and *C*.
[b] Infusions discontinued after 11 consecutive daily injections.
[c] Extent of hydropic degeneration in convoluted tubules: present = +; moderate = 2+; extensive = 4+.
[d] Additional: adrenal, striated muscle, and pancreas essentially normal throughout.

Histological sections from the orally fed controls (Fig. 1, *A*) also appeared essentially normal and were omitted from Table II in which histological changes in OPG-infused animals that died are shown. Histological changes in lymphoid tissue due to irradiation were observed in animals that died one and one-half to five hours after injections began. A single death three days postirradiation showed: acute passive congestion in the liver, diffuse hepatitis (characterized by central lobular necrosis), vacuolization of the hepatic nuclei, diffuse areas of pneumonia with alveolar collapse in the lung, and hydropic degeneration in the kidney convoluted tubule epithelium. These findings became intensified and included hemorrhage in the lung, heart, stomach, and intestines of animals that died seven to thirteen days postirradiation but were somewhat diminished in a single animal that died on the nineteenth postirradiation day. The histological changes and gross findings at autopsy were similar to those observed previously in irradiated tapioca-dextrin-infused rabbits (9).

DISCUSSION

Initial deaths (25%, one to five hours) and the high mortality rate during the injection period (62%, one to eleven days) were similar to what was observed in irradiated rabbits receiving repeated intravenous infusions of tapioca dextrin (9). Early deaths were attributed to cardiac failure whereas others (12–13) have reported initial shock-like deaths in irradiated noninfused rabbits. The terminal convulsion and vascular findings observed mitigate against a shock-like death. Intravenous infusions herein administered should restore any decreased circulating blood volume anticipated from reported postirradiated fall in blood pressure after 600 to 800 r (13). Initial deaths did not occur in the irradiated noninfused controls, in nonirradiated tapioca-dextrin (11) or OPG-infused rabbits (10). Our results would appear to indicate that irradiation may have sensitized the rabbits to the infusion fluid or decreased their ability to compensate for the large increase in circulating fluid volume, resulting in cardiac failure, the mechanism for which remains undetermined.

Later deaths were accompanied by hypervolemia, edema, infection, gastrointestinal hemorrhage, and hydropic degeneration in the kidney tubule epithelium. The hypervolemia and edema were probably the result of the large volume of fluid infused, while the infection and hemorrhage were probably related to irradiation injury. The hydropic degeneration in the convoluted tubule epithelium is a characteristic of intravenously injected "plasma substitutes" (14) and is not due to irradiation. However, multiple OPG replacement infusions in dogs were reported to have minimal deleterious effects (15) indicating that there is a species difference in the deleterious effects produced by "plasma substitutes."

SUMMARY AND CONCLUSIONS

1. Irradiation increased the sensitivity and the mortality rate of rabbits given large and repeated intravenous infusions of oxypolygelatin solution, confirming an earlier observation in which tapioca dextrin solution was similarly used.

2. Initial deaths (one to five hours after starting infusions) were attributed to cardiac failure. Later deaths (three to nineteen days postirradiation) were accompanied by hypervolemia, edema, infection, hemorrhage, and hydropic degeneration in the convoluted tubules. These effects appeared following three to seven daily infusions, were increased in intensity after seven to eleven injections, and became less pronounced seven days after infusions were discontinued. Irradiated noninfused control animals all survived and showed only minor histological evidence of irradiation damage when sacrificed thirty days postirradiation.

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Quaternary Salts of 2,4,6-(Dialkylaminoalkoxy)-1,3,5-Triazines and 2,4-(Dialkylaminoalkoxy)-Quinazolines as Curare Agents*

By JOHN R. HOHMANN and JAMES W. JONES†

Three 2,4,6-(dialkylaminoalkoxy)-1,3,5-triazines, two 2,4-(dialkylaminoalkoxy)-quinazolines, seven *tris* quaternary salts of the triazines and three *bis* quaternary salts of the quinazolines were prepared and identified. Seven of the quaternary salts were tested for curariform activity by the mouse screen-drop test and the rabbit head-drop test. In the chick, the quaternary salts produced a flaccid paralysis associated with a pachycurare-type block. Of the seven compounds tested, six were appreciably less active than Flaxedil®. One compound, the tribenzchloride of 2,4,6-(β-diethylaminoethoxy)-1,3,5-triazine, was approximately twice as active as Flaxedil® in the mouse and seven times as active in the rabbit.

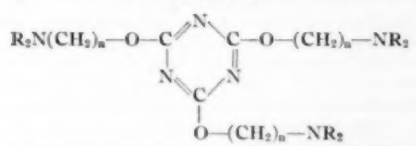
SINCE Bovet's (1) success in synthesizing a potent curare agent using the supposed structure of *d*-tubocurarine as a guide, other groups have investigated a large number of compounds for their curare activity. Included among the curariform agents studied by Bovet were the phenylcholine ethers, one of which exhibited a potent action in the rabbit (2). This compound, tri-(β-triethylammoniumethoxy)-benzene-1,2,3-triiodide (Flaxedil®), has been used in the clinic. Winter and Lehman (3) studied a similar group of phenolic ethers which included the diethiodide of *p*-di(β-dimethylaminoethoxy)benzene (diethamine) and the diethiodide of *p*-di(γ-dimethylaminopropoxy)benzene (dipropamine).

In the present study, two series of compounds, namely the quaternary salts of 2,4,6-(dialkylaminoalkoxy)-1,3,5-triazines and the quaternary salts of 2,4-(dialkylaminoalkoxy)-quinazolines, were synthesized and their curariform activities determined in the mouse and rabbit.

EXPERIMENTAL

2,4,6-(Dialkylaminoalkoxy)-1,3,5-Triazines.—A modification of the method of Spielman, *et al.* (4), was used to prepare these compounds. One-half mole of the appropriate amino alcohol (β-diethylaminoethanol, β-dimethylaminoethanol or γ-diethylaminopropanol) was mixed with an equal volume of dry benzene. Six and nine-tenths grams (0.3 mole) of sodium metal was added in small pieces to the cooled solution. The reaction mixture was refluxed until the sodium was completely reacted. Eighteen and four-tenths grams (0.1 mole) of cyanuric chloride[1] was dissolved in 150 ml. of dry benzene and added slowly to the sodium alkoxide solution. The mixture was kept cold during this addition. The mixture was then refluxed for one hour and filtered while hot. The filtrate was washed with water, then dried over sodium sulfate. Most of the benzene was removed by distillation at atmospheric pressure. The remaining benzene and amino alcohol were removed under reduced pressure. Two distillations of the final product were necessary to obtain an analytically pure sample. Boiling points, yields, and analyses are shown in Table I.

TABLE I.—2,4,6-(DIALKYLAMINOALKOXY)-1,3,5-TRIAZINES

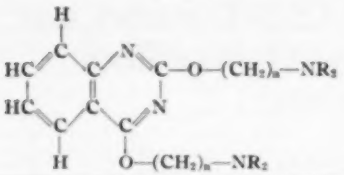


R	n	Boiling Point, °C.	Yield, %	N, % Calcd.	N, % Found
CH_2CH_3	2	195–197 @ 3 mm.	39	19.72	19.79
CH_2CH_3	3	245 @ 4 mm.	36	17.71	17.35
CH_3	2	183–185 @ 3 mm.	27	24.56	24.89

2,4-(Dialkylaminoalkoxy)-Quinazolines.—The method described in *Organic Syntheses* was used to prepare benzoylene urea. The method of Lange, *et al.* (5), was used to prepare 2,4-dichloroquinazoline. A suspension of sodium aminoalkoxide was prepared by reacting 2.6 Gm. (0.12 mole) of sodium with 0.18 mole of the appropriate alcohol (β-diethylaminoethanol, β-dimethylaminoethanol or γ-diethylaminopropanol) in 50 ml. of dry benzene. Twelve grams (0.06 mole) of crude 2,4-dichloroquinazoline was added, keeping the reaction mixture cooled in ice. The mixture was then refluxed for two hours, cooled, washed with water, and dried over sodium sulfate. The benzene was removed by distillation at atmospheric pressure. The remaining mixture was distilled twice under reduced pres-

* Received October 24, 1953, from the State University of Iowa College of Pharmacy, Iowa City.
An abstract of a dissertation submitted by John R. Hohmann to the Graduate College, State University of Iowa, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Pharmacy.
† Professor of Pharmacy, State University of Iowa, College of Pharmacy.
[1] Supplied by the American Cyanamid Company.

TABLE II.—2,4-(DIALKYLAMINOALKOXY)-QUINAZOLINES



R	n	Boiling Point, °C.	Yield, %	N, % Calcd.	N, % Found
CH_3CH_2	2[a]	225–226 @ 4 mm.	54	15.55	15.51
CH_3CH_2	3	230–233 @ 2 mm.	40	14.43	14.28
CH_3	2	234–236 @ 9 mm.	35	18.42	18.07

[a] Previously reported in literature.

sure to obtain the pure amine. Boiling points, yields, and analyses are shown in Table II.

Quaternary Salts of 2,4,6-(Dialkylaminoalkoxy)-1,3,5-Triazines.—The triethiodides were prepared by reacting the amine with ethyl iodide in a Parr bomb. The amine (0.01 mole) and 0.05 mole of ethyl iodide were thoroughly mixed in the bomb cup. The bomb was sealed and heated to 100° for twenty-four hours. The quaternary salt formed was dissolved in absolute alcohol with the aid of heat. The salt was precipitated by adding absolute ether to the cooled alcoholic solution. The precipitate was collected, washed with anhydrous ether, and dried in a vacuum oven at 60°.

The trimethiodides could not be prepared in the Parr bomb since mixing the amine and methyl iodide resulted in a violent reaction. The following method was found to be satisfactory. The amine (0.01 mole) and 0.05 mole of methyl iodide were refluxed in 50 ml. of absolute alcohol for two hours. Absolute ether was then added to precipitate the quaternary salt. The precipitate was collected, washed with anhydrous ether and dried in a vacuum oven at 60°.

The tribenzchloride was prepared by the method described for the triethiodides.

Yields, melting points, and analyses are shown in Table III.

Quaternary Salts of 2,4-(Dialkylaminoalkoxy)-Quinazolines.—In preparing these salts, it was necessary to determine whether or not the nitrogen atoms in the pyrimidine portion of the molecule quaternized (Chart I).

To study this problem, 2,4-diethoxyquinazoline was prepared (5) and reacted with alkyl iodides under various conditions. When the quinazoline was refluxed with methyl iodide in absolute alcohol for two hours, only the unreacted quinazoline was recovered. When the quinazoline and ethyl iodide were reacted in a Parr bomb, a solid substance was formed which was insoluble in water, alcohol, or ether. The substance was not characterized; however, it did not contain the starting materials or the quaternary salt. The compounds desired in this study were those having only the quaternized side chain amino groups. Quaternization was effected by refluxing the amine (0.01 mole) with 0.03 mole of methyl iodide in 50 ml. of absolute ethanol for two hours. The quaternary salt was precipitated by the addition of absolute ether. The precipitate was collected, washed with absolute ether and dried in a vacuum oven at 60°. Yields, analyses, and melting points are shown in Table IV.

Pharmacological Procedures.—*Chick Test.*—A large number of substances have been classified as curarizing agents. However, the mechanism of action of many of these compounds differs radically from *d*-tubocurarine. Bovet, *et al.* (6), have proposed a classification of most curare agents into two groups, namely, the pachycuraries which are believed to act by blocking the acetylcholine depolarizing action on the motor end plate and the leptocuraries which act by producing a depolarizing action on the motor end plate. In the bird, the leptocuraries produce contracture and rigidity of the muscles while the pachycuraries give a flaccid paralysis similar to that produced in the mammal. The chick test was used in this study to determine the probable mode of action of the experimental compounds. All the compounds in the quinazoline and triazine series were found to produce the flaccid paralysis associated with a pachycurare-type block.

Potency Tests.—In previous investigations of curariform drugs the most active and inactive members of the series were the trimethylammonium or triethylammonium compounds. For this reason only

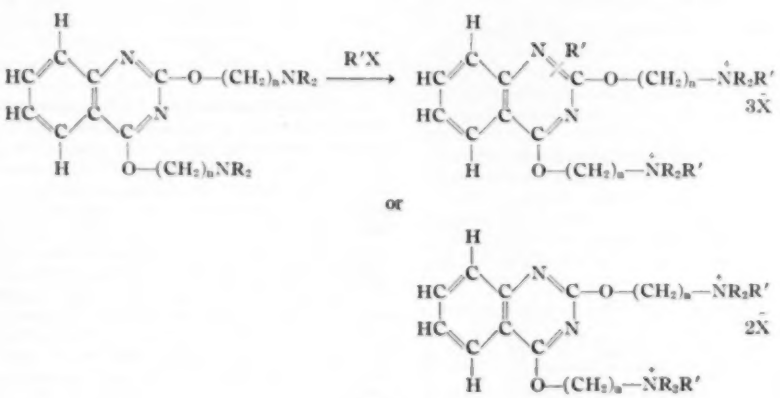
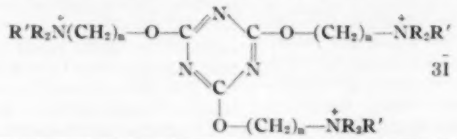


Chart I

TABLE III.—TRIS QUATERNARY SALTS OF 2,4,6-(DIALKYLAMINOALKOXY)-TRIAZINES



R	R'	n	Yield,[a] %	Melting[b] Point, °C.	I, % Calcd.	I, % Found	N, % Calcd.	N, % Found
Me	Me	2	84	296–298	49.46	49.19	10.94	10.94
Me	Et	2	72	271–273	47.01	46.41	10.37	10.27
Et	Me	2	81	248–250	44.69	43.89	9.86	9.73
Et	Et	2	76	243–244	42.60	41.85	9.39	9.23
Et	Et	3	76	251–253	40.69	40.20	8.96	8.76
Et	Me	3	86	249–250	42.60	42.45	9.39	9.49
Et	Benzyl	2	80	151–154	13.22[c]	13.06[c]	10.44	10.27

[a] Based on amine used in quaternization. [b] Decomposition in all compounds. [c] Per cent chlorine instead of iodine.

TABLE IV.—BIS-QUATERNARY SALTS OF 2,4-(DIALKYLAMINOALKOXY)-QUINAZOLINES

R	R'	n	Yield, %	Melting Point,[a] °C.	% I Calcd.	% I Found	% N Calcd.	% N Found
Et	Me	2	95	247–248	39.45	39.17	8.69	8.67
Me	Me	2	83	299–301	43.17	42.32	9.52	9.32
Et	Me	3	62[b]	...	37.78	36.90	8.34	8.19

[a] Hygroscopicity caused difficulty in purification. [b] Decomposition in all compounds.

the most highly methylated and ethylated members of a given series were screened. The one tribenzchloride derivative was also included in the potency tests.

A modification of the screen-drop test described by Cavillito, *et al.* (7), and the rabbit head-drop test (8) were used to determine the relative potencies of the compounds. In the mouse screen-drop test, groups of 10–20 white mice were used at each dosage level. The ED_{50} and its fiducial limits were determined using the method of probit analysis described by Burn (9). The agents were administered intraperitoneally in 0.5 ml. of solution.

In the rabbit head-drop method, groups of six animals were used. The solutions were adjusted to give a head-drop within 1–2 ml. The results of the mouse sloping-screen test and of the rabbit head-drop test are shown in Table V.

Toxicity Test.—The high potency of the tribenzchloride of tris-(β-diethylaminoethoxy)-1,3,5-triazine (compound 7) made a preliminary study of its toxicity of interest. The toxicity was determined on three groups of 15 white mice, using the probit method to determine the LD_{50}. The LD_{50} of compound 7 was 5.05 mg./Kg. The fiducial limits (P = 0.95) of the LD_{50} were 4.69–5.36 mg./Kg.

TABLE V.—PARALYZING DOSES IN THE MOUSE SLOPING-SCREEN AND RABBIT HEAD-DROP TESTS

Drug	Mouse Sloping-Screen Test, mg./Kg.	Fiducial Limits, P = 0.95	Rabbit Head-Drop Test, mg./Kg.	Fiducial Limits, P = 0.95
(*1*) 2,4,6-(β-diethylaminoethoxy)-1,3,5-triazine triethiodide	63.00	60.23–65.40	6.29	5.78–6.80
(*2*) 2,4,6 - (β - dimethylaminoethoxy)1,3,5 - triazine trimethiodide	135.90	128.10–143.40	58.06	35.76–80.36
(*3*) 2,4,6 - (γ - diethylaminopropoxy) - 1,3,5 - triazine triethiodide	15.61	14.43–16.70	6.34	5.47–7.21
(*4*) 2,4 - (β - diethylaminoethoxy) - quinazoline dimethiodide	19.07	18.71–20.77	3.17	2.19–4.15
(*5*) 2,4 - (β - dimethylaminoethoxy) - quinazoline dimethiodide	36.98	33.17–40.88	12.36	7.76–16.96
(*6*) 2,4 - (γ - diethylaminopropoxy) - quinazoline dimethiodide	7.42	6.76–7.95	2.99	1.99–3.99
(*7*) 2,4,6 - (β - diethylaminoethoxy)-1,3,5-triazine-tribenzchloride	2.12	1.88–2.48	0.0552	0.0337–0.0777
(*8*) Flaxedil ®	4.12	3.84–4.39	0.391	0.325–0.456

DISCUSSION

The relative activities of the experimental compounds are found to differ in the mouse and rabbit. This is undoubtedly due to species variations which have been previously reported for curare agents (10). In the triazine series, the *tris* benzyl derivative (compound 7) is definitely the most active compound. It is approximately twice as active as Flaxedil® in the mouse and seven times as active as Flaxedil® in the rabbit. The tris-triethylammoniumethoxy and tris-trimethylammoniumethoxy triazines (Nos.1 and 2) are relatively inactive. In the mouse, No. 1 is approximately $^1/_{15}$ as active as Flaxedil® while its methyl homolog (No. 2) is $^1/_{30}$ as active as Flaxedil®. In the rabbit, No. 1 is approximately $^1/_{12}$ as active as Flaxedil® while No. 2 is only $^1/_{100}$ as active. A similar decrease in activity is found in the methyl homolog of Flaxedil®. In compound No. 3, the alkyl side chains were lengthened by one methylene group. This compound was four times as active as compound No. 1 in the mouse, but in the rabbit no significant difference in activity was noted. Compound No. 3 was less active than Flaxedil® in both the mouse and the rabbit.

A comparison of the N to N distance in these compounds is of interest. Fischer-Hirschfelder models were constructed and the N to N distances measured with the nitrogen atoms at maximum and equal distances apart. The three nitrogens in compounds Nos. 1, 2, and 7 will describe an equilateral triangle of 11.5 Å. side length. Compound No. 3 will describe an equilateral triangle of 14 Å. side length. If one considers the 15 Å. N to N distance proposed by several workers for optimal curare activity, the low activity of Nos. 1 and 3 is unexpected. In Flaxedil®, the onium head distances, when at maximal and equal distances, describe a triangle of 10 Å. side length. The experimental compounds more closely approach the proposed 15 Å. for optimum activity and might be expected to be relatively active compounds. The nature of the triazine ring, although isosteric with benzene, or the symmetrical arrangement of the onium heads within the molecule may play a greater role than onium head distances. In this light, the preparation and study of the benzene isoster of compound No. 1 would be of interest.

The compounds prepared in the quinazoline series appear to be more active than the corresponding members of the triazine series. The replacement of ethyl by methyl groups in the quinazoline series also resulted in a decrease in activity. Compound No. 5 is only $^1/_2$ as active as No. 4 in the mouse and only $^1/_4$ as active in the rabbit. Increasing the length of the alkyl side chain in the quinazolines has much the same effect as in the triazine series. Compound No. 6, whose N to N distance is 16 Å., is approximately 2 to 3 times more active in the mouse than No. 4, whose N to N distance is 13 Å. In the rabbit, there is no significant difference in the potency of these compounds.

A continuation of this investigation is in progress.

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Symposium on Antibiotics

THE SECOND ANNUAL SYMPOSIUM ON ANTIBIOTICS will be held on October 20, 21, and 22, 1954, in the Auditorium of the U. S. Department of Health, Education, and Welfare, 4th Street and Independence Avenue, Washington, D. C. The symposium is sponsored by the Division of Antibiotics of the U. S. Department of Health, Education, and Welfare and the journal *Antibiotics and Chemotherapy*.

Topics suggested for presentation at the Symposium are: new clinical and experimental facts on tetracycline; the combined use of hormones and antibiotics; new developments in broad spectrum therapy, including parenteral therapy; the combined clinical use of antibiotics and other chemotherapeutic agents; prophylactic uses of antibiotics; the use of antibiotics in pediatrics; antibiotics in the animal feed industry, veterinary medicine, and agriculture; new facts on penicillin and streptomycin; antibiotics and chemotherapeutics in TB; and new laboratory methods for antibiotics.

In addition to contributors of papers, it is planned to invite a few outstanding clinicians in the field to speak on timely topics related to their specialties.

Study of a Special System of Transportation in the Epicarps of *Urticaceae**

By EGIL RAMSTAD

The epidermis of the mature fruits of most *Urticaceae* is filled with mucilage. The mucilage develops from the bottom of the cells of the epicarpic layer and thus isolates the layer from the rest of the pericarp. Yet the individual cells of this layer still are able to maintain communication with the subjacent layers of the pericarp through a special system of communication. This system consists of cells, named intercalary cells, distributed at even intervals throughout the pericarp. The intercalary cell is devoid of mucilage, and every mucilage cell touches at one point or other, one and only one, intercalary cell. The exchange of metabolites and the transport of constructive material from the mesocarp for the formation of the mucilage take place through the intercalary cell.

IN A PREVIOUS paper (1) the author has shown the existence of regularly distributed, non-mucilaginiferous cells in the mucilaginous epicarps of many species of the Nettle family. These cells have been overlooked by previous investigators who have studied the structure of the pericarps of the Nettle family. Since, in a sense, these structures are unique in the plant world, additional and detailed information about them is reported in this paper.

DESCRIPTION

General Structure of the Pericarp of Urticaceous Plants.—All the *Urticaceae* which I have investigated possess a pericarp composed of four layers (Fig. 1). In most species the endocarp consists of strongly undulated and strongly lignified walls (Fig. 2). Rather frequently one finds also that the inner wall of the inner mesocarpic layer is lignified. The lignification extends halfway out on the radial wall (Figs. 1, 3). The lignified areas are strongly pitted. In the species in which the described layers are lignified (which is true in most species), a rosette crystal of calcium oxalate is present on the bottom of the lignified portion of the inner mesocarp layer. The crystal is attached to the wall by help of a fine sack of cellulose, in which it is enclosed (Fig. 1). The outer layer of the mesocarp consists of smaller cells, and, like the epicarp, contains chloroplasts (Fig. 3).

Isolation of the Epicarpic Cell by Mucilage Formation.—When the epicarpic layer approaches its mature size, the formation of mucilage is initi-

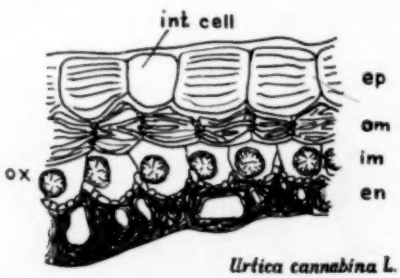


Fig. 1.—*Urtica cannabina* L.—Transverse section of pericarp.[1]

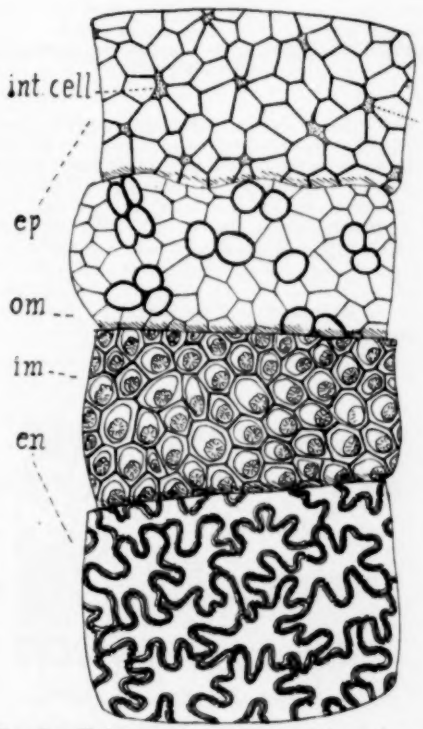


Fig. 2.—*Urtica urens* L.—Tangential section of successive layers of pericarp.

* Received August 21, 1953, from the Purdue University School of Pharmacy, Lafayette, Ind.
Presented to the Scientific Section, A.PH.A., Salt Lake City meeting, Utah, August, 1953.

[1] Abbreviations used in figures: *en*—endocarp; *ep*—epicarp; *im*—inner mesocarp; *int. cell*—intercalary cell; *muc.*—mucilage; *om*—outer mesocarp; *ox*—oxalate crystal.

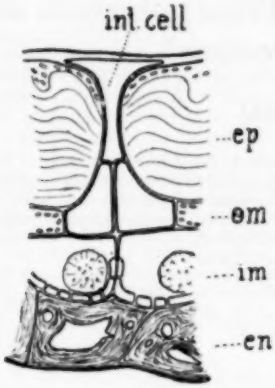


Fig. 3a.—*Urtica urens* L.—Transverse section of the pericarp, showing detailed structure of an intercalary cell.

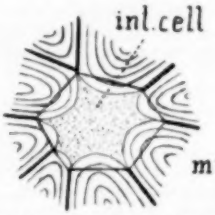


Fig. 3b.—*Urtica urens* L.—Surface view of an intercalary cell.

ated. In the mucilage-forming species the epicarpic as well as the outer mesocarpic cells are at that stage all gorged with single or compound starch grains of varying sizes (Fig. 4). The calcium oxalate crystals of the inner mesocarp layer are then laid down. At this time a fine hyaline layer appears on the bottom of the epidermal cells, which constitutes the initiation of mucilage formation by those cells. The quantity of starch present decreases proportionally as the amount of mucilage increases. When all the starch originally present in the outermost layers of the pericarps is consumed, the mucilage has reached the maximum of development and, in most cases, nearly fills the entire epicarpic cells. During the later stages of mucilage deposition the endocarpic layer becomes strongly lignified.

Many seeds possess a mucilaginous epidermis However, in all such cases the mucilage is deposited on the *outer wall* of the cell and consequently grows in thickness in a centripetal direction. In the epicarp of mucilage-forming fruits of the Nettle family, however, the mucilage is deposited in centrifugal direction on the *inner wall* of the epicarpic cell (Figs. 3, 4).

As a result of the presence of a mucilage bed on the inner wall of the epicarpic cells, the latter should become isolated and incapable of exchanging metabolites with the subjacent layers. Soon the cells should be unable to produce more mucilage for lack of constructive material, and the cell, incapable of the exchange of metabolites, should die. One finds, however, that the mucilage does form in such quantities that the epidermal cells get filled with it (Fig. 3). The depots of starch observed in the subepidermal layer prior to the mucilage formation disappear during the laying-down of the mucilage. A

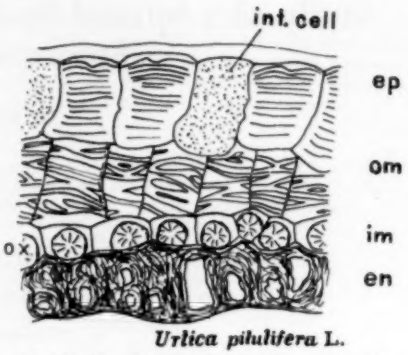


Fig. 5.—*Urtica pilulifera* L.—Transverse section of mature pericarp.

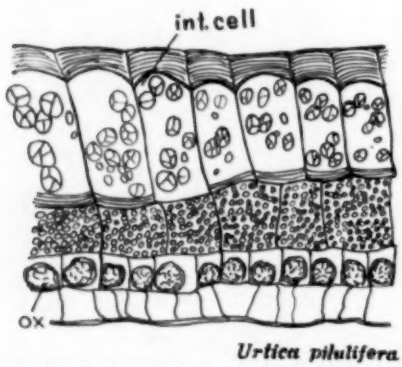
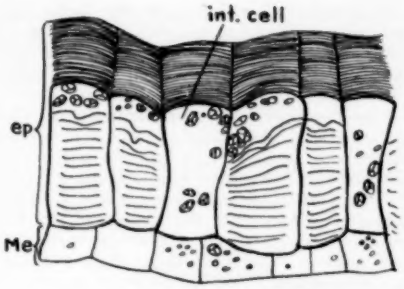


Fig. 4.—*Urtica pilulifera* L.—Transverse section of young pericarp.

Fig. 6.—*Urtica membranacea* L.—Surface view of epicarp of mature fruit, showing reinforcement of the walls of the intercalary cells.



Urtica pilulifera L.

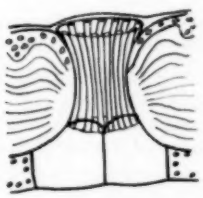
Fig. 7.—*Urtica pilulifera* L.—Transverse section of epicarp and outer mesocarp during the mucilage deposition.

system, therefore, must exist which mediates the transport of metabolites from the lower layers into the epicarpic cells. This system consists of some special cells scattered evenly throughout the entire epicarp. Because of their location between the mucilage cells, I have designated these cells as *intercalary cells*.

The Intercalary Cells.—The intercalary cells are mostly inconspicuous and are easily overlooked by examining the pericarps of urticaceous plants.

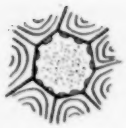
At an early stage of development, the intercalary cell is indistinguishable from the other epicarpic cells except by the fact that it is richer in proteins (Fig. 4, *ep*). This can be demonstrated by immersing sections of young fruits in protein colorants. It is possible by this procedure to discern the intercalary cells by the deeper color of their content. One also notices that they are regularly distributed over the surface of the fruit (Figs. 2, 6, 11). Most often there is one intercalary cell for every seven or eight mucilage cells. Often in the mature pericarp the intercalary cells can be distinguished from the mucilage cells by their smaller size (Figs. 3, 8, 10), by the presence of special reinforcing structures on their walls (Figs. 5, 8–10, 12), or sometimes by a deeper hue. The intercalary cells differ fundamentally from the other epicarpic cells in being devoid of mucilage. Consequently they can serve as cells mediating the transport of metabolites from the mesocarp into the surrounding mucilage-producing cells of the epicarp after the deposited mucilage has made the inner epicarpic cell wall unfit for transport of metabolites across it.

During the early stages of mucilage formation the intercalary cell is in communication with the mucilage cell throughout the entire radial wall (Fig. 4). However, as the mucilage fills the epicarpic cells more and more by deposition on the bottom, the extent of the radial wall which remains free for communication between the mucilage cells and the



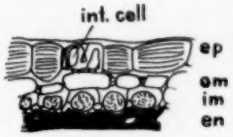
Urtica dioica L.

Fig. 8.—*Urtica dioica* L.—Transverse section through epicarp and outer mesocarp of mature fruit showing columnar reinforcement of the intercalary cell.



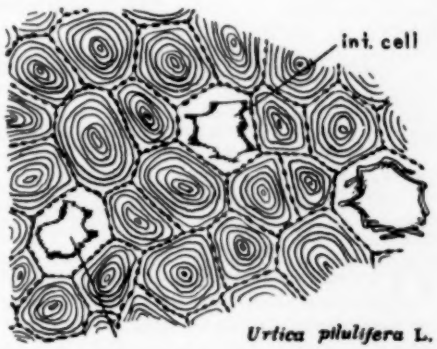
Urtica dioica L.

Fig. 9.—*Urtica dioica* L.—Surface view of intercalary cell with wall reinforcement.



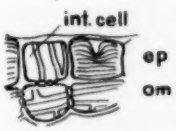
Urtica membranacea L.

Fig. 10.—*Urtica membranacea* L.—Transverse section of pericarp.



Urtica pilulifera L.

Fig. 11.—*Urtica pilulifera* L.—Surface view of epicarp with intercalary cells.



Urtica cannabina L.

Fig. 12.—*Urtica cannabina* L.—Reinforced intercalary cell and reinforced cells of the outer mesocarpic layer.

intercalary cell diminishes gradually. At full maturity of the fruit, the mucilage cells are nearly completely filled; but there will always remain a small area of the outer portion of the radial wall which is free of mucilage and through which the communication with the intercalary cell is assured (Figs. 3, 7, 8). This small area is covered with protoplasm as long as the cell is alive.

Due to the pressure exerted by the developing mucilage, the radial walls of the mucilage cells tend to bulge into the intercalary cell, making its lumen quite small (Figs. 3*a*, 3*b*). In species with heavy mucilage formation, the intercalary cells may be fortified to resist obliteration by the pressure from the mucilage cells through the development of special, radially or tangentially arranged, columnar reinforcements on the radial walls (Figs. 6, 8, 12). In some species the reinforcement has assumed the form of a network around the radial walls (Fig. 10).

In radial or transversal sections of certain fruits one notices that the intercalary cells are shorter than the mucilage cells (Fig. 3). To compensate for the short size, two or more of the subjacent cells of the outer mesocarpic layer are elongated radially while their sister cells remain flattened. In some species these same cells are colored more deeply than their neighboring cells and thereby become easily discernible. Protein reagents also show them to be richer in proteins than their equals. It is evident that they also are involved in a vigorous transport of plastic material to the mucilage-forming cells.

It is noteworthy that each mucilage cell touches one intercalary cell only. In the case of *Urtica membranacea* L., however, a mucilage cell may occasionally border more than one intercalary cell (Fig. 6). Apparently, the intercalary cell is of no physiological significance in the species of the Nettle family which are devoid of mucilage formation in their epicarp. But even in mucilage-free species they are present.[2] The coloring of tangential sections with protein colorants brings these cells out clearly. In some fruits their existence can be assessed from the deeper color of evenly distributed cells of the outer layer of the mesocarp, since a darker color of these cells is also often observed in species with normal mucilage-containing epicarp.

[2] E. g., *Pilea pumila* (L.) A. Gray, *Debregesia longifolia* (Burm.) Wedd., *Girardiania palmata* Gaud, *Urticastrum divaricatum* (L.) Kunze.

REFERENCE

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A Study of the Behavior of Morphine on Ion Exchange Resins*

By EUGENE E. HAMLOW, H. GEORGE DeKAY, and EGIL RAMSTAD

The behavior of morphine on several ion exchange resins was determined primarily with the objective of selecting resins which might be suitable for quantitative work with morphine. The choice of resins was tentatively evaluated by applying them in the separation of morphine from known alkaloidal mixtures and opium tincture. A comparison of the ultraviolet spectra of the material isolated from the mixtures with the spectrum of pure morphine indicated that small amounts of nonalkaloidal impurities were present in the morphine fraction.

One of the main difficulties associated with the determination of morphine in opium or its preparations is that of separating morphine from the other opium alkaloids and extraneous extractives. Once morphine is isolated in a sufficiently pure state, the quantitative determination is relatively easy.

In theory, ion exchange offers a unique method of overcoming the difficulties of separating morphine from a variety of accompanying substances. Because of the basic character of morphine, one ought to be able to separate it from neutral and acidic substances by use of proper cationic resins; and since morphine is also phenolic in nature, it should be picked up by strong anionic resins, which would have no attraction for nonphenolic alkaloids.

Ion exchange resins as well as sulfonated coals have been used for the isolation and concentration of nicotine, quinine, scopolamine, atropine, and ephedrine (1–3). Jindra (4) has made use of a weakly basic resin (Amberlite® IR-4B) to convert a number of alkaloidal salts to the corresponding free bases, which then could be titrated.

After the present work was terminated, Levi and Farmilo (5) published a paper which, in the main features, concurs with the results of Jindra (4). Grant and Hilty (6) have recently

* Received August 21, 1953, from Purdue University School of Pharmacy, Lafayette, Ind.
Presented to the Scientific Section, A. Ph. A., Salt Lake City meeting, August, 1953.

published a procedure for the separation of morphine and codeine by use of a strongly basic resin.

The purpose of this paper is to report the behavior of morphine toward various ion exchange resins. Primarily, we wanted to find out which resins might offer promise for use in quantitative determination of morphine in complex mixtures of alkaloids and other constituents.

EXPERIMENTAL

Ion Exchange Resins and Apparatus.—The following resins were tested:

Amberlite® IR-120 A.G., strong cationic exchanger.
Amberlite® IRC-50 A.G., weak cationic exchanger.
Amberlite® IRA-400 A.G., strong anionic exchanger.
Amberlite® IR-4B A.G., weak anionic exchanger.
Duolite® C-60, weak cationic exchanger.
Ionac® A-300, anionic exchanger of intermediate strength.
Nalcite® SAR, strong anionic exchanger.

Three sizes of columns were used: (*a*) microcolumns, 0.3 x 20 cm., consisting of 2-ml. graduated pipets filled with approximately 2 ml. of resin, held in place by a grain of sand and operated at a flow rate of 10 drops per minute; (*b*) semimicro columns, 1 x 7–8 cm., operated at 20 to 25 drops per minute, and (*c*) 3 x 15-cm. columns, operated at 30 to 40 drops a minute.

Spectrophotometric Determination of Morphine.—Since traces of materials dissolve from the resin columns and appear in the eluate with morphine, the measuring of morphine by determining its absorption of light in the ultraviolet failed, because the resin materials also absorbed the ultraviolet light. We therefore used the nitrosomorphine color reaction as modified by Adamson and Handisyde (7), for the quantitative determination of morphine. Since the acidity of the reacting solution is of some importance, all fractions, whether acidic or alkaline, were neutralized if necessary and evaporated to dryness in 50-ml. Erlenmeyer flasks *in vacuo* in a "dish and dome" vacuum distilling apparatus on a steam bath. The residue was dissolved in 0.1 *N* HCl, and an aliquot portion pipetted into a 50-ml. volumetric flask, reagent added, and the flask filled to the mark. The per cent transmittancy of the color was determined after fifteen minutes on a Beckman Model DU spectrophotometer at 442 mμ (1-cm. cell). A calibration curve for morphine was prepared, and the concentration of the solution, expressed in milligrams of anhydrous morphine, was read from it. The method was determined to be accurate to within ±2%. Other quantitative and qualitative determinations in the ultraviolet were carried out with the same instrument.

Behavior Studies.—The size of the columns was varied and, according to the kind of resin used, their particle size was also varied. The columns were backwashed in order to classify the resin bed hydraulically, regenerated with an excess of regenerant (10% HCl for cationic resins and 4% Na_2CO_3 or NaOH for anionic resins), and washed with water until free of the regenerant. A solution containing a known amount of the alkaloid was passed and thoroughly washed through the column and the effluent was assayed in order to determine the extent of the exchange. The columns were then eluted with a chosen elutriant, 25-ml. fractions were collected, and each fraction assayed to determine the character of the elution process. The efficiency of the elutriant was visualized by graphing the relationship of the "concentration of the fraction" to the "volume of eluate."

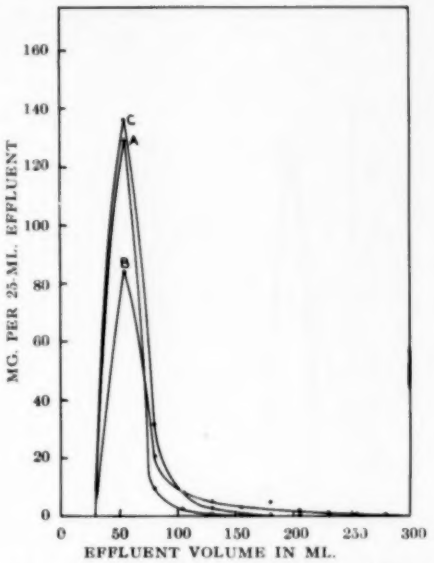

Fig. 1.—The elution of morphine from Amberlite® IR-120. *A*—148 mg. eluted with 4 *N* methanolic NH_3. *B*—141 mg. eluted with 4 *N* HCl. *C*—188 mg. eluted with 1 *N* NaOH.

The amount of morphine added to each column was not held constant. An aliquot of the morphine solution to be used in each column run was assayed in order to determine accurately the quantity of morphine used. These amounts are indicated for each elution curve in the figures. In the studies of the quantitative behavior of the resin the effluent was not fractionated, and a volume of elutriant was used which previously had been found to remove all of the morphine.

Amberlite® IR-120.—Morphine from aqueous solutions of morphine sulfate was adsorbed quantitatively by this resin. Several elutriants were tried: ammoniacal methanol, eluting the morphine as the free base; aqueous HCl, eluting the morphine as the hydrochloride; and aqueous NaOH, eluting the morphine as the soluble sodium morphinate. The eluting efficiency of 4 *N* methanolic NH_3, 4 *N* aqueous HCl, and 1 *N* NaOH is shown in Fig. 1. Complete recovery of the morphine was obtained by use of 4 *N* methanolic NH_3. The particle size of the resin did not have an appreciable effect on the rate of elution, as is evident from Fig. 2. The operation of a 30–40 mesh column was satisfactory.

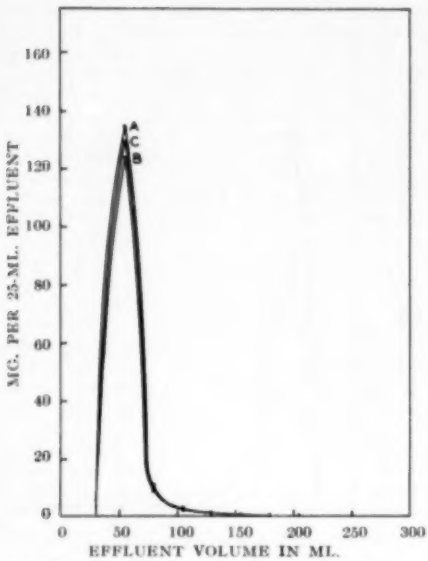

Fig. 2.—The effect of the particle size of Amberlite® IR-120 on morphine elution. Elutriant—4 *N* methanolic NH_3. *A*—156 mg. on 40–60 mesh resin. *B*—145 mg. on 30–40 mesh resin. *C*—148 mg. on 20–40 mesh resin.

Amberlite® IRC-50.—As expected, neither the sodium nor the hydrogen form of the resin was able to split morphine sulfate effectively enough to bring about an adsorption of morphine on the column. However, when a methanolic solution of morphine base was passed through the column (H-form), the morphine was adsorbed quantitatively. Semimicro columns were employed in order to avoid serious packing in the column, for a swelling of the resin particles results when the morphine is adsorbed. A large-diameter column and shallow bed allowed the bed to expand upward. Quantitative elution of the morphine from the resin could be achieved both with 1 *N* HCl and 4 *N* methanolic NH_3. The course of the elution processes with these two elutriants is shown in Fig. 3.

Higher normality of HCl (4 *N*) was less effective as an elutriant, probably because it closes the pores in the outer portion of the resin particles more rapidly (shrinkage), thereby impairing the free movement of the morphine ion. Recovery with 1 *N* HCl as an elutriant was found to be 99.3%, 4 *N* NH_3 eluted 99.4%.

Duolite® C-60.—Experiments were carried out to test its ability to adsorb morphine base. The results indicated that the resin did not exhibit enough capacity for morphine for our purpose.

Amberlite® IR-4B.—The suitability of this resin for use in preparing free alkaloids from alkaloidal salts has been demonstrated by Jindra (4).

The resin splits the salts of morphine readily. Due to the low solubility of the liberated free morphine base, the alkaloid crystallizes out in the eluate, and sometimes partly in the resin bed. It was therefore necessary to use an organic solvent to elute the morphine base. The morphine sulfate therefore, was, dissolved in 75% methanol. The solution was passed through the column and the liberated morphine was washed out with methanol and determined both colorimetrically and titrimetrically. The larger semimicro columns were washed after regeneration until the effluent was neutral to methyl red. Quantitative removal of the sulfate ion from solution was obtained with this procedure. Recovery of morphine was good as shown in Table I.

TABLE I.—RECOVERY OF MORPHINE

Fraction	Volume, ml.	Total Morphine (Colorimetric), mg.	Total Base (Titrimetric), mg.
1	100	257.0	255.0
2	100	3.6	8.0
3	200	3.0	10.9
Total		263.6	273.9
Per cent recovered		99.3	103.0

Ionac® A-300.—The experiments with Ionac® A-300 paralleled those with Amberlite® IR-4B. When morphine sulfate was passed through the column, it was observed that not only was the sulfate ion adsorbed, but also morphine was retained partially by the resin (70–73%). The part of morphine that was adsorbed could be removed with 1 *N* HCl. The resin, then, exhibits sufficient alkalinity to react with the phenolic group of the morphine base formed from the reaction of the sulfate ion with the resin. As a result of this behavior, the resin was judged nonadaptable for quantitative separations of morphine.

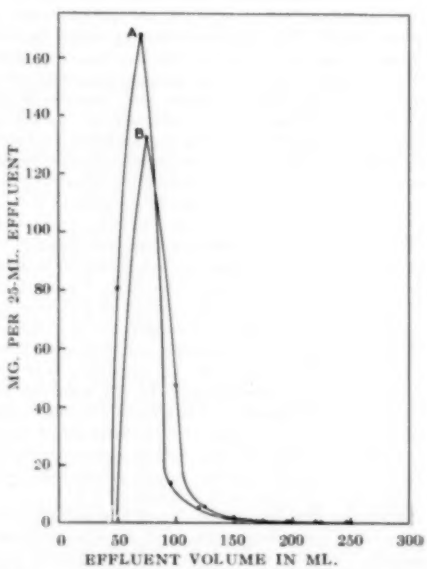

Fig. 3.—The elution of morphine from Amberlite® IRC-50. *A*—192.5 mg. eluted with 1 *N* HCl. *B*—192 mg. eluted with 4 *N* methanolic NH_3.

Amberlite® IRA-400.—This resin removed the morphinate ion completely from a methanolic solution of morphine alkaloid. The morphine could be eluted quantitatively with 1 *N* HCl. It was necessary to employ semimicro columns in order to facilitate the removal of gas pockets which formed in the resin bed when the columns were eluted with HCl. The course of the elution process with 1 *N* HCl is shown in curve *4A*, Fig. 4.

Nalcite® SAR.—Experiments with this resin were similar to those carried out with Amberlite® IRA-400. The resin removed morphine quantitatively from a methanolic solution of the alkaloid. It was more difficult to achieve effective elution of morphine from this resin than from Amberlite® IRA-400. The character of the elution process is shown in curve *4B*, Fig. 4.

Separation of Alkaloidal Mixtures.—The resins which behaved favorably in regard to morphine adsorption and elution were used in attempts to separate morphine from other alkaloids. We chose papaverine and narcotine, and the complex mixture of alkaloids and other compounds present in tincture of opium. Particular interest was attached to the use of narcotine since this alkaloid, which is present in opium, is a lactone, which structure may open in the presence of strong base, and, as a result, contaminate the morphine fraction.

Two combinations of resins were employed: (*a*) Amberlite® IR-4B and IRA-400, and (*b*) Amberlite® IR-120 and IRA-400. The relative purity of the morphine isolated was empirically determined by visual comparison of the U. V. absorption spectra of the isolated material with that of pure morphine.

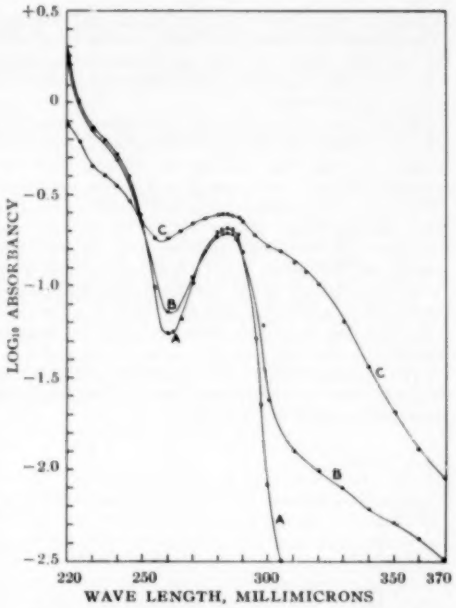

Fig. 5.—Ultraviolet spectra of the material isolated by use of the IR-4B-IRA-400 procedure. *A*—Pure morphine-reference curve. *B*—"Morphine" eluate from known mixture. *C*—"Morphine" eluate from opium tincture.

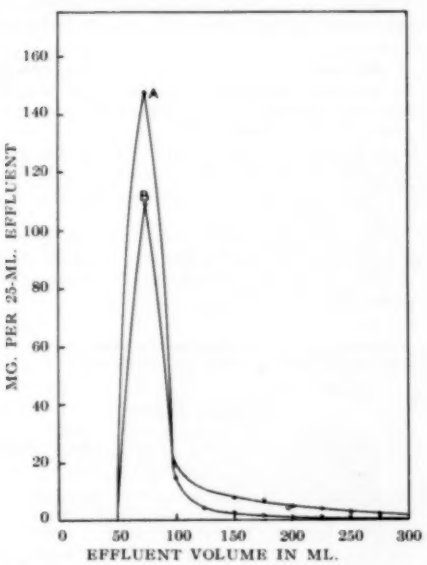

Fig. 4.—The elution of morphine from Amberlite® IRA-400 and Nalcite SAR. *A*—180 mg. eluted from Amberlite® IRA-400 with 1 *N* HCl. *B*—188 mg. eluted from Nalcite SAR with 4 *N* HCl.

Amberlites® IR-4B and IRA-400.—The larger semimicro columns were employed. IR-4B resin was used as the first resin to adsorb all anions and convert the alkaloids to free bases. As second resin the IRA-400 resin was chosen, since it would adsorb from among the bases only those bases which contain phenolic groupings. The two columns were connected in series.

Morphine sulfate, papaverine hydrochloride, and narcotine hydrochloride dissolved in 75% methanol were passed through the columns in quantities of 200–500 mg. of each. The columns were subsequently washed with 500 ml. of 75% methanol. The IRA-400 column was eluted with 300–500 ml. of 1 *N* HCl, and the U. V. absorption spectrum of the recovered material determined. Approximately 95% of the morphine was recovered (colorimetric method). The spectrum is shown as curve *5B*, Fig. 5. Curve *5A* is the spectrum of pure morphine.

The procedure was repeated with opium tincture diluted with 3 volumes of methanol. The final effluent was still colored. The free base was determined in aliquots of the effluent from each column by electrometric titration. The titration indicated that IR-4B removed only the stronger acids, and that the more strongly basic IRA-400 removed the weaker acids together with the phenols, thus causing contamination of the isolated morphine with weak acids. The spectra of the material eluted from the IRA-400 column are shown as curve *5C*, Fig. 5.

Amberlites® IR-120 and IRA-400.—The larger semimicro columns were employed also in the experiments with these resins. The totality of the

alkaloids was adsorbed on the IR-120 (*H*) column. The column was eluted, the eluate concentrated and passed through the column of IRA-400 to adsorb the phenolic alkaloids. The adsorbed alkaloids could then be eluted.

An aqueous solution of morphine sulfate, papaverine hydrochloride, and narcotine hydrochloride was passed through the column of IR-120, and the column washed with water and with methanol. The bases were eluted with 4 *N* methanolic NH_3 and the methanol evaporated. The residue was dissolved in methanol and washed through the column of IRA-400. The IRA-400 was eluted with 1 *N* HCl and the U. V. spectrum for the isolated material determined. The spectrum is shown as curve *6B*, Fig. 6. Curve *6A* represents that of pure morphine.

The same procedure was repeated with opium tincture, and the spectrum of the isolated material is shown as curve *6C*, Fig. 6.

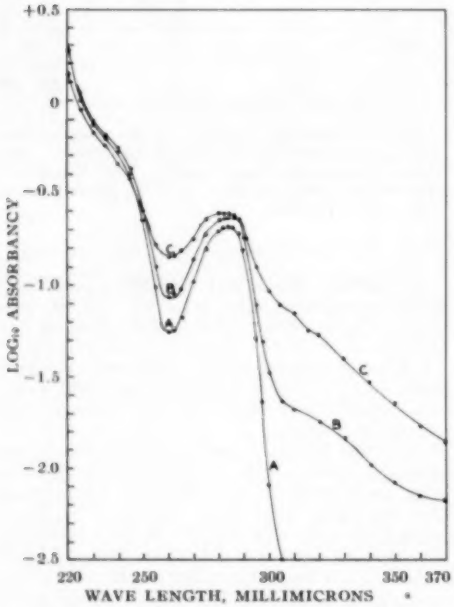

Fig. 6.—Ultraviolet spectra of the material isolated by use of the IR-120-IRA-400 procedure. *A*—Pure morphine-reference curve. *B*—"Morphine" eluate from known mixture. *C*—"Morphine" eluate from opium tincture.

DISCUSSION AND SUMMARY

The results from the preceding investigations show that some of the available ion exchange resins have properties which make them fitted for quantitative work with morphine and its salts.

Amberlite® IR-120 picks up morphine quantitatively, and by use of 4 *N* methanolic NH_3 the alkaloid can be recovered completely with a minimum of elutriant. On the other hand, Amberlite® IRC-50, whether in hydrogen or sodium form, is not able to split the salts of morphine to an extent that makes it suited for quantitative work. However, it is well fitted to pick up morphine base, and recovery is nearly quantitative when 1 *N* HCl or 4 *N* NH_3 is used as elutriants.

The findings of Jindra (4) as to the efficiency of Amberlite® IR-4B as an adsorbant for the sulfate ion of morphine sulfate were verified. The alkaloid in aqueous solution may crystallize out partly on the resin but can be eluated quantitatively with methanol.

Amberlite® IRA-400 retains morphine from an alkaline solution and consequently is able to separate morphine from nonphenolic alkaloids.

Other resins which were tested possessed certain characteristics which made them less fitted for quantitative work with morphine.

Amberlite® IRA-400 can pick up morphine because of the presence of the phenol hydroxy group on the alkaloid. The resin was shown not to hydrolyze the lactone group of alkaloids of the narcotine type. It is therefore possible to separate phenolic alkaloids from all other alkaloids present in opium by this resin. The quantity of phenolic alkaloids in opium other than morphine is of the order of 1% of the amount of morphine and, therefore, is of little or no significance for the determination of morphine by use of ion exchange resins.

The morphine isolated from known mixtures of alkaloids could not be found to contain other alkaloids, but yet was not quite pure as evidenced from the ultraviolet adsorption spectrum. The impurities derived from substances thrown off from the resin columns themselves during the adsorption and elution.

Unfortunately, with the simple setup reported above for isolation of morphine from opium tincture, some coloring matter passes through the column and appears in the eluate with morphine. This fact shows up by a greater distortion of the ultraviolet absorption spectrum of the isolated morphine than of that resulting from pure morphine passed through the same columns.[1]

From the established data, the prospect of establishing a method for an exact morphine determination in opium preparations by use of ion exchange resins is promising.

[1] Experiments not yet terminated show that the color can largely be eliminated by additional procedures.

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Study of Possible Complex Formation Between Macromolecules and Certain Pharmaceuticals*

III. Interaction of Polyethylene Glycols with Several Organic Acids

By TAKERU HIGUCHI and JOHN L. LACH

A study has been made of the complexing behavior of high molecular weight polyethers with a number of pharmaceutical compounds. By means of solubility procedures it has been found that although pentobarbital and barbital have little or no tendency to complex with polyethylene glycols, phenobarbital forms stable and stoichiometric molecular compounds with these macromolecular substances. Phase analysis indicates that one phenobarbital molecule is bound by two ethylene oxide units of the polyether chains. It is also shown by another phase analytical method that phenolic compounds are bound by the polyethers in the same fashion, the higher molecular weight polymers exhibiting greater complexing tendency than those of lower degrees of polymerization. Experimental data obtained on the various acids investigated indicate that these compounds are only weakly bound.

IN EARLIER STUDIES from these laboratories it was shown that substances such as caffeine (1–4) and polyvinylpyrrolidone (5, 6) form both soluble and insoluble molecular complexes with a large number of acidic compounds in aqueous solution. Theoretical considerations of these systems have led to the conclusions that this type of complex formation is far more widespread than was commonly realized. On the basis of this hypothesis it was postulated that polyethers such as polyethylene glycol (Carbowax®[1]) and other related compounds can also be expected to form similar complexes in water. The results of theoretical and experimental exploration of this predicted behavior are presented in this communication.

THEORY

The complexes of concern in these studies are of relatively weak molecular variety. They possess much smaller energy of interaction than those of Mulliken (7), since the state of molecular orbitals is essentially that of parent molecules, the electron exchange type of interaction probably playing a comparatively minor role in the formation of these complexes. The energy of reaction in these systems appears to be derived mainly from the dipole-dipole type interaction of the reactants and secondarily from the "squeezing out" effect of water. The nature of the two contributions will be discussed in detail in the following paragraphs.

The dipolar interaction of the xanthines, polyamides, ketones, etc., with compounds containing acidic hydrogen is due principally (6) to the presence of polarized carbonyl groupings in these compounds. Their high solubility in water, for example, is evidently due to hydrogen bond (dipole-dipole interaction) formation with water molecules. Since phenols, barbiturates, carboxylic and amino compounds possess acidic hydrogen capable of coordinating with negative centers, these substances can replace water molecules from the carbonyl grouping. The extent of such replacement reactions in aqueous solutions, on the other hand, would be extremely small on the basis of the dipolar interactions alone since the competing water dipoles are present in such overwhelmingly high relative concentration.

The replacement reaction is rendered favorable, however, by the interaction of the hydrophobic, lipophilic portions of the interacting molecules. The intermolecular attractive tendencies of these organic groupings are relatively small, but in a sense these hydrophobic units are "squeezed out" of the water phase by the high internal pressure of water leading to a relatively high degree of interaction. Since squeezing together of the hydrocarbon portions of the reactants permits the water molecules originally surrounding these units to form additional hydrogen bonds, the net effect is an apparent high degree of interaction between the relatively inert hydrocarbon groups. All this results in a very appreciable increase in the stability of these complexes. Similarly, other water-soluble organic compounds such as the polyethers may be expected to form complexes with organic acids, the point of attachment for the ethers being the basic ether oxygen.

Another factor which is probably highly influential in determining the relative stability of these complexes is the stereochemical relationship of the reactant. It is evident that the degree of interaction can be greatly modified by minor variation in the shapes of the reacting entities. Thus, for example, although polyethylene glycol interacts significantly with *p*-hydroxybenzoic acid, polypropylene glycol had no tendency to form a complex with this acid.

PAST WORK

Very little attention has been paid to the possible existence of complex formation between polyethers such as Carbowax® and pharmaceutical compounds. Much of early evidence on the complexing

* Received February 18, 1954, from the School of Pharmacy, University of Wisconsin, Madison.
Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.
[1] Carbowax® is a registered trademark of Carbide and Carbon Chemicals Corp., New York, N. Y.

behavior of synthetic macromolecules has been restricted to that on polyvinylpyrrolidone. Pellerat, *et al.* (8, 9), have reported evidence for such an interaction for PVP based on pharmacological data. Quantitative studies by Higuchi and Kuramoto (5, 6) confirmed the existence of such PVP complexes in solution.

Although polyethylene glycols have seen considerable usage in pharmaceutical formulations, limited direct investigations of the possibilities of complex reactions by these compounds appear to have been made. Water-insoluble coordination complexes of the polyglycols and heteropoly inorganic acids (silicotungstic, phosphomolybdic, etc.) have been known for some time. Possible complex formation by these PEG systems, furthermore, with many pharmaceutical compounds (phenol, tannic acid, iodine, salicylic acid) are indicated by reports of incompatibilities in these systems (10–13). No serious direct investigation of complex formation with organic compounds appears to have been made.

EXPERIMENTAL OBSERVATIONS ON PHENOBARBITAL, BARBITAL, AND PENTOBARBITAL

The solubility method was employed in determining the extent and nature of possible complexing reactions between the several barbiturates and polyethylene glycols. This method is quite suited to studies of this type since it is sensitive to such phenomena and does not depend on the isolation of unstable products. In the procedure the effect of varying concentrations of a complexing agent on the solubility of the drug in solution is studied. If complexing takes place to an appreciable degree, one observes either an increase or a decrease in the solubility depending on the nature of the complex formed. The phase diagrams obtained from such studies provide, furthermore, a quantitative means of calculating the stoichiometry of the reaction.

Phenobarbital appears to interact strongly with all the glycols studied as is evident from Figs. 1, 2, 3, and 4, showing the effects of varying polyethylene glycol (PEG) concentration on the apparent solubility of a fixed quantity of the barbiturate in a definite volume of solution. The PEG concentration was calculated on a repeating unit basis, that is, gram equivalents of ethylene oxide linkage ($CH_2—O—CH_2$) per liter. The marked decrease in the amount of phenobarbital in solution caused by added PEG as shown in these phase diagrams is indicative of complex formation. The complex formed in this system appears to be extremely insoluble in nature, since no initial increase in the apparent solubility of phenobarbital was observed as was the case in the caffeine-barbiturate interactions (4).

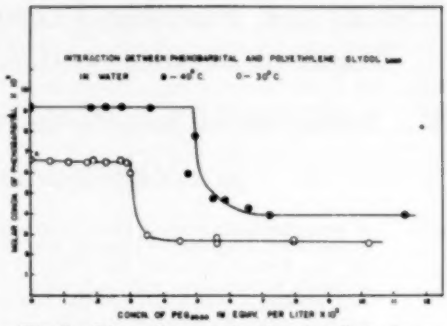


Fig. 2.—Phase diagram showing the effect of varying concentrations of PEG 6000 on the apparent solubility of phenobarbital.

Analogous to other similar systems (3, 4) it is seen that the total concentration of phenobarbital in the plateau region A–R of these phase diagrams (Figs. 1 and 2) is an invariant independent of the amount of PEG since any phenobarbital precipitated in form of the complex is replaced by further dissolution of the solid free barbiturate. At point *R* the system is no longer saturated with respect to the phenobarbital since all of the excess solid barbiturate has been converted over to the complex. Further addition of PEG results in the depletion of free phenobarbital in solution.

The stoichiometric ratio of the components of the phenobarbital-PEG complex formed in the plateau region can be calculated from these phase diagrams.

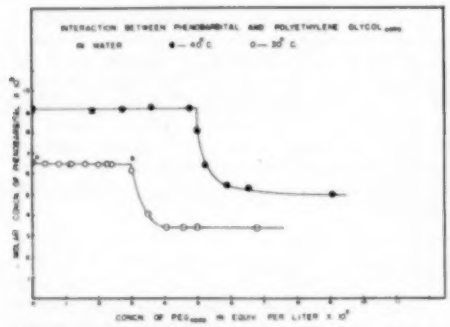


Fig. 1.—Phase diagram showing the effect of varying concentrations of PEG 4000 on the apparent solubility of phenobarbital.

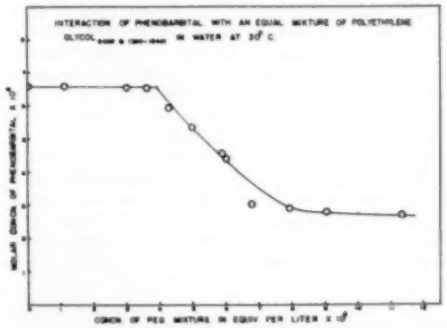


Fig. 3.—Phase diagram showing the effect of varying concentrations of an equal mixture of PEG 6000 and (300–1540) on the solubility behavior of phenobarbital.

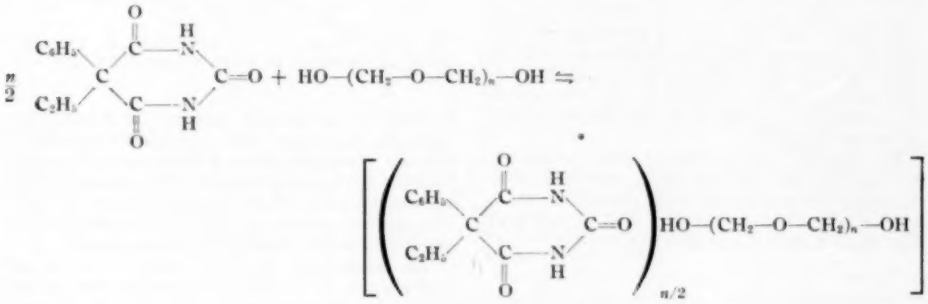
This is possible because, if the above analysis is true, the amount of PEG represented by the plateau region is equal to that entering into the complex during this interval and the corresponding amount of phenobarbital being converted to the complex is equal to that present in form of free undissolved solid at point A. Thus for phenobarbital at 30°:

PEG content of complex formed in plateau region = 30×10^{-3} equiv./liter (from diagram, Fig. 1)
Phenobarbital content of complex = Total phenobarbital added to system − phenobarbital in solution at point A
= $21.5 \times 10^{-3} - 6.5 \times 10^{-3} = 15 \times 10^{-3}$ moles/liter

Then the stoichiometric ratio equals

$$\frac{\text{PEG content}}{\text{Phenobarbital content}} \quad \frac{30 \times 10^{-3}}{15 \times 10^{-3}} = 2$$

The results indicate that the complex is of a 2:1 type, two ethylene oxide units reacting with one phenobarbital molecule. This 2:1 type of interaction can be represented by the following equation:



where n is equal to the number of ethylene oxide linkages present in the molecule. The value n for PEG 4000, for example, is approximately equal to 90. Assuming this equation to obey the mass law, we can write the stability constant k,

$$k = \frac{[(C_{12}H_{12}N_2O_3)_{n/2}HO{-}(CH_2{-}O{-}CH_2)_n{-}OH]}{[(C_{12}H_{12}N_2O_3)_{n/2}HO{-}(CH_2{-}O{-}CH_2)_n{-}OH]}$$

This relationship can explain the observed high-phenobarbital low-PEG dependency of this interaction. In all probability, however, the equation is not governed strictly by the mass law relationship. Some deviation can be expected on the basis of a cooperative effect, the phenobarbital molecules interacting with the ether centers being influenced by other phenobarbital molecules already attached to the chain.

The lower molecular weight polyethylene glycols do not appear to interact with the barbiturate as strongly as the higher members of the series. The amount of free phenobarbital in equilibrium with the solid complex in solution is greater in these lower molecular weight glycols as shown in Figs. 1, 2, and 3. The unreactivity of the lower glycols is apparent even in mixtures of polyethylene glycols as is evident in Fig. 3 which shows the interaction of phenobarbital and an equal mixture of PEG 6000 and PEG (300–1540), the latter being a blend of equal parts of polyethylene glycol 300 and 1540.

If it is assumed that a 2:1 type of interaction takes place it is possible to show by means of the phase diagram shown (Fig. 3) that PEG 300 in the glycol mixture probably does not complex with phenobarbital. As previously pointed out, the plateau region of the diagram represents the amount of PEG entering into complex formation during this interval and the corresponding amount of phenobarbital being converted to the complex is equal to that present in form of free undissolved solid in the system at zero PEG concentration. In order to maintain the 2:1 stoichiometric ratio only three-quarters of the PEG present in the plateau region must interact, that is, PEG 300 representing one-quarter of the total mixture does not interact with the barbiturate to any appreciable extent. Thus,

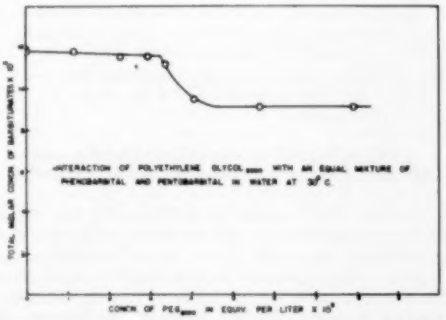


Fig. 4.—Influence of varying amounts of PEG 4000 on the apparent solubilities of an equal mixture of barbiturates.

PEG content of complex formed in plateau region = $^3/_4 \times 40 \times 10^{-3} = 30 \times 10^{-3}$ equiv./liter (from diagram)

Phenobarbital content of complex = Total phenobarbital added to system − free phenobarbital in solution
$= 21.5 \times 10^{-3} - 6.5 \times 10^{-3} = 15 \times 10^{-3}$ moles/liter

The stoichiometric ratio equals

$$\frac{\text{PEG content}}{\text{Phenobarbital content}} \quad \frac{30 \times 10^{-3}}{15 \times 10^{-3}} = 2$$

As shown in Fig. 5 barbital and pentobarbital unlike phenobarbital gave no evidence of interaction with PEG 4000. Even in mixtures with phenobarbital, pentobarbital did not appear to undergo interaction to any appreciable extent. This lack of reactivity of pentobarbital, for example, cannot be attributed to any lack of "seeds" or nuclei of reaction since even during *in situ* formation of phenobarbital-PEG complex no significant amount of pentobarbital complex appears to undergo coprecipitation. This was evident from a solubility experiment carried out on a mixture of the two barbiturates, the results being shown in Fig. 4. The initial barbiturate concentration in the diagram corresponds to the sum of the solubilities of the two components, but the break in the diagram corresponds to reaction only with the phenobarbital, the entire curve being identical with that obtained for phenobarbital alone as shown in Fig. 2 except for the amount of barbiturate in solution contributed by the pentobarbital.

The negative results obtained with barbital and pentobarbital are probably due in part to a stereochemical effect and in part to the absence of the aromatic ring in these compounds; such a group lends itself more readily to complex formation.

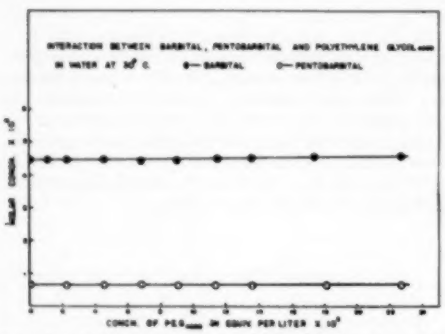


Fig. 5.—Solubility behavior of barbital and pentobarbital in presence of PEG 4000.

EXPERIMENTAL OBSERVATIONS ON RESORCINOL, CATECHOL, AND PHENOL

As was noted by earlier workers (10, 13), PEG even in relatively low dilution reacts with phenolic compounds to yield quite water-insoluble oily liquids. The extent and nature of this reaction, unfortunately, could not be followed by the solubility method employed for the barbiturates because of the high water solubility of the reactants and the apparent indefinite composition of the product formed. Since the reaction product is a liquid, however, a considerable amount of information regarding the system can be gained by miscibility studies. Thus the extent of these reactions can be followed by titrating aqueous PEG solutions with aqueous phenolic solutions to cloud point, the relative amounts of the reactants being indicative of the stoichiometric dependency of the reaction.

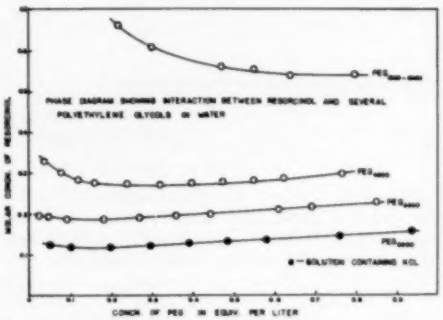


Fig. 6.—Influence of several PEG's on the solubility of resorcinol at room temperature (28° C.).

The interactions of several polyethylene glycols with resorcinol, catechol, and phenol studied in this manner are presented in Figs. 6 and 7. Areas above the lines in these phase diagrams represent regions of immiscibility of the phenol-PEG complex-rich layer with the water-rich layer, whereas below the lines we have completely homogeneous systems. Analogous to the phenobarbital-PEG systems, the lower molecular weight glycols did not appear to interact as strongly as the higher members of this series, as is evident from these phase diagrams. As shown in Fig. 6 the critical concentration of resorcinol at the cloud point increased markedly with a decrease in molecular weight of the interacting gly-

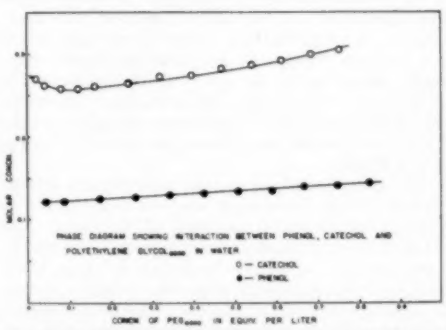


Fig. 7.—Influence of PEG 6000 on the solubility of phenol and catechol at room temperature (28° C.).

col. The critical phenolic concentrations appeared to be only slightly temperature-dependent since temperature changes did not appreciably affect their values. The observed high-phenolic low-PEG dependency can probably be explained in a fashion similar to that of the phenobarbital interaction although the stoichiometry of this phenol-PEG reaction does not seem to be as clearly defined. Attempts to characterize the resorcinol-PEG 6000 complex gave a relationship of 1:6 with respect to resorcinol and the glycol.

In all probability reactions of this type are of highly cooperative nature similar to such phenomena as melting and dissolution of solids. The individual tendencies of phenol molecules to attach themselves to a polyether molecule are relatively small but when several of them do combine with the ether chain a favorable environment for additional reaction is created.

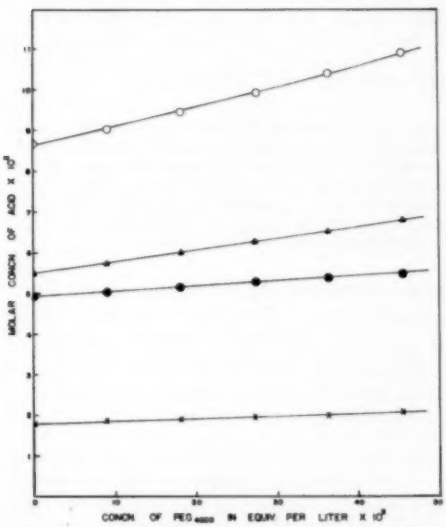


Fig. 8.—Phase diagram showing the effect of varying concentrations of PEG 4000 on the apparent solubility of several organic acids in water at 30° C. ○, *m*-hydroxybenzoic acid. ▲, *p*-hydroxybenzoic acid. ●, *o*-phthalic acid. *X*—salicylic acid.

OBSERVATIONS ON *m*-HYDROXYBENZOIC ACID, *p*-HYDROXYBENZOIC ACID, SALICYLIC ACID, AND *o*-PHTHALIC ACID

The interactions between several organic acids and polyethylene glycol 4000 were studied by means of the solubility method. The effect of PEG on the equilibrium amount of acid in solution is shown in Fig. 8. It is evident that the extent of complex formation in these systems is relatively very small compared to that of the preceding cases. It is rather surprising that the introduction of a carboxyl group on a phenol molecule should influence the complexing tendency to such a marked degree. This effect can be partly rationalized on the basis that the "squeezing out" action of water, previously referred to, is not as fully effective in these systems since the acidic molecules have been rendered more hydrophilic by the presence of the carboxyl groups.

EXPERIMENTAL PROCEDURE

Barbiturates.—*Reagents.*—Recrystallized phenobarbital U. S. P., m. p. 174–175°; recrystallized barbital U. S. P., m. p. 189.5–190.5°; recrystallized pentobarbital (prepared from sodium pentobarbital U. S. P.), m. p. 129–130°; PEG 4000, 6000, and (300–1540), Carbide and Chemicals Co.; 0.02 *N* alcoholic sodium hydroxide; thymolphthalein and alizarine yellow as indicators.

Procedure.—The experimental procedure and method of analysis employed here were essentially those used in the caffeine-barbiturate system (4).

Excess quantities of the barbiturate were placed in 125-ml. glass-stoppered bottles together with varying amounts of PEG and 50 ml. of distilled water was added to each. The bottles were placed in a mechanical shaker in a constant temperature bath and equilibrated for sixteen hours. Aliquot portions of the supernatant liquid were removed and analyzed for the barbiturate. Phenobarbital and barbital were determined by microtitration with alcoholic sodium hydroxide (14) with thymolphthalein as the indicator for phenobarbital and alizarine yellow for barbital. Pentobarbital was analyzed spectrophotometrically. Excess quantities of phenobarbital, 250 mg. at 30° and 400 mg. at 40°, were used in these determinations; for barbital 1.0 Gm., pentobarbital 200 mg., and 250 mg. each of phenobarbital and pentobarbital were used for the mixture of barbiturate.

Phenols.—*Reagents.*—Recrystallized resorcinol U. S. P., m. p. 110–111°; recrystallized catechol Matheson Co., m. p. 104–105°; reagent grade phenol; 5% aqueous solutions of PEG 4000, 6000, and (300–1540).

Procedure.—Distilled water was added to each titration flask containing varying amounts of the PEG solutions (1–10 ml.) so that the final volume in each was 10 ml. These were titrated at room temperature (28°) with 10% aqueous solutions of resorcinol, catechol, and a 5% aqueous solution of phenol to cloud points. Calculation of molar concentrations of the phenolic compounds and molar equivalents of PEG were based on total volume of solution in the titration flasks.

Organic Acids.—*Reagents.*—Recrystallized *m*-hydroxybenzoic acid Eastman Kodak, m. p. 201–202°; recrystallized *p*-hydroxybenzoic acid Eastman Kodak, m. p. 214–215°; recrystallized salicylic acid U. S. P., 159°; *o*-phthalic acid, m. p. 205–207°; 0.5 *N* barium hydroxide solution; bromthymol blue and phenophthalein as indicators.

Procedure.—The solubility method, as that employed for the phenobarbital-PEG system, was used except for the following differences:

A weakly acidic (0.003 *N*) sulfuric acid solution was employed as the solvent to suppress any acid dissociation. The bottles were equilibrated for fourteen hours at 30° after which time aliquot portions were removed and titrated with barium hydroxide solution for the acid content. Excess quantities of the acids, *p*-hydroxybenzoic (1.0 Gm.), *m*-hydroxybenzoic (0.40 Gm.), salicylic (0.40 Gm.),

and *o*-phthalic (1.0 Gm.), were used in these studies. Bromthymol blue was the indicator for the hydroxybenzoic acids and phenolphthalein for *o*-phthalic acid.

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Salicylamide I. Absorption, Excretion, and Blood Levels*

By JAMES M. CRAMPTON† and ELBERT VOSS‡

An investigation of salicylamide blood levels in rabbits showed that maximum levels are reached in one hour falling rapidly thereafter, until the blood is practically free of drug in three to four hours. Absorption is rapid from the gastrointestinal tract. Excretion is also rapid, 81 per cent being recovered in the urine in four hours. The method of analysis employed is essentially that of Brodie, Udenfriend, and Coburn for salicylate, but preceded by acid hydrolysis. The hydrolytic treatment is believed to remove the glycuronide, freeing the reactive phenolic hydroxyl group.

ALTHOUGH salicylamide has been known for a long time, it has attracted little attention until recent years. Aspirin and other salicylates have enjoyed an uninterrupted popularity for many years. The efficacy and relative lack of toxicity of the salicylates have given them a degree of acceptability unrivaled by any other groups of drugs. However, the large dosage used in the treatment of rheumatic fever and other rheumatic diseases is attended by varying degrees of toxicity. Nausea and vomiting, and tachypnea are among the most frequent toxic manifestations of large doses of salicylate and are signs of a central nervous system stimulation. Thus a drug with the analgesic, antirheumatic action of salicylate, but without central stimulating action, would seem to be a valuable addition to the field of therapy. Salicylamide appears to be such a drug.

Ichniowski and Heuper (1), Hart (2), and Litter, *et al.* (3), have reported salicylamide to be depressant rather than stimulating to the central nervous system. They found the acute toxicity in animal work to be but slightly greater than aspirin and the chronic toxicity to be very much less. Clinical trials reported by Litter, *et al.* (3), indicate fewer side effects.

Seeberg, Hansen, and Whitney (4) published a report on the absorption and distribution of salicylamide. Using the direct method of Keller (5) for determination of salicylates in blood serum, they found very low blood levels in rats and rabbits after administration of very large doses. Ichniowski and Heuper (1), using the method of Brodie, Udenfriend, and Coburn (6) for determination of salicylate in blood plasma, had reported no salicylate in blood samples after large doses of salicylamide to dogs. Euler and Remy (7), however, using the method of Thoburn and Hanzlik (8) preceded by acid hydrolysis in four hours of refluxing, found higher blood levels in humans than with corresponding doses of salicylate. These authors stated that the ferric nitrate method of Brodie and of Keller did not always show the same sensitivity with salicylamide and that conversion of salicylamide to salicylate was necessary before it could be analyzed.

* Received October 30, 1953, from the College of Pharmacy, University of Florida, Gainesville.
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Bray and co-workers (9, 10) have reported the isolation of several metabolic products of salicylamide after giving the drug to rabbits. They state that whereas salicylic acid is eliminated principally as ether-soluble acid, salicylamide is excreted principally as the ether glucuronide. Mandel, Rodwell, and Smith (11), in a report on the metabolism of salicylamide labeled with C^{14}, state that the major portion is eliminated as ethereal glycuronide. No salicylic acid metabolites were found in this investigation on human patients. Brodie and Szekely (12) report that under conditions simulating those of the intestine salicylamide is not hydrolyzed.

EXPERIMENTAL

Analysis of Salicylamide in Blood

Investigation of the ferric nitrate salicyl reaction showed that salicylamide will react with the ferric ion to produce the characteristic violet color of the iron salicyl reaction. The concentration recommended by Brodie, *et al.* (6), however, did not produce as intense a color as desired. Increasing the iron concentration to 0.55% gave a deeper color, practically equivalent to that of salicylic acid. Other modifications of the Brodie method with respect to quantities of reagents used gave us an analytical procedure in which salicylamide or salicylate gave a color reaction whose intensity was proportional to the salicyl concentration and which was sensitive to 1 mg. %.

Application of this method to blood samples taken at various times after administration of salicylamide failed, however, to reveal more than a trace of salicylamide after doses of 100–300 mg./Kg. In view of the work of Bray (9, 10) and of Seeberg (4), and since we had established an analytical procedure by which salicylamide could be recovered quantitatively from blood serum when added in known amounts, we considered the failure to recover salicylamide from the serum of animals to which large doses had been given to be due to the conjugation of the reactive phenol group of salicylamide with glycuronic acid *in vivo*.

Test of blood samples of rabbits, which had received salicylamide using the Euler-Remy method in which blood samples were subjected to hydrolysis before analysis, did reveal salicyl which had not been detected by the direct analysis. However, this method which calls for four hours of refluxing followed by steam distillation was laborious, time-consuming, and not suitable for small samples of blood. The analytical procedure of these authors was therefore modified so as to eliminate the steam distillation step and substitute instead the ethylene dichloride extraction procedure used by Brodie.

The method of analysis of salicylamide as finally amended and adopted in our experimental work follows:

Reagents.—*1.* Hydrochloric acid 2 *N*. *2.* Ethylene dichloride. *3.* Ferric nitrate solution 1% in 0.07 *N* HNO_3. *4.* Ferric nitrate test solution, 0.55% in 0.038% HNO_3. Made by adding to 50 cc. of ferric nitrate solution distilled water to make 90 cc.

Procedure.—*1.* To 1 cc. blood serum add 1 cc. 2 *N* HCl. *2* (*a*). Seal the acidified serum in a glass ampul and place in boiling water bath for six hours; or (*b*) place the acidified serum in a small flask and reflux while boiling gently for three hours; or (*c*) if free salicylamide only is to be determined, omit step *2* altogether. *3.* To the hydrolyzed serum, add 10 cc. ethylene dichloride and shake for five minutes. *4.* Centrifuge the mixture for five minutes and remove the supernatant layer by aspiration. *5.* Decant 9 cc. of the ethylene chloride phase and add it to 15 cc. of ferric nitrate test solution. *6.* Shake vigorously for three minutes, then centrifuge for three minutes. *7.* Pour the clear supernatant colored solution into a spectrophotometer tube and read the optical density at 540 millimicrons. *8.* Determine the salicyl content by comparison with a standard curve previously prepared.

Preparation of Standard Curve.—A standard curve was prepared by making standard solutions of salicylamide and sodium salicylate representing 50 mg. salicylic acid per 100 cc. Suitable aliquots of these solutions were diluted and treated as described and the optical densities used to plot a standard curve. Salicylamide and sodium salicylate gave practically identical results. The method is therefore valid even if part of the salicylamide is hydrolyzed to salicylic acid in the preliminary treatment.

Analysis of Salicylamide in Urine.—The same method of analysis was used in determination of salicylamide in urine. In most cases it was necessary to dilute the urine sample to bring it within the analytical range. The usual dilution was one to twenty, but this may be altered at will and the salicylamide content calculated accordingly. There is slight reaction from normal urine due to the presence of phenolic substances, but since the salicylamide content is high and the urine is diluted the effect of interfering substances can be ignored.

Specificity.—The basic test employed in this analysis is the iron-salicyl reaction, a well-established analytical procedure. The probable sources of error in our analytical procedure would lie in the destruction of the salicyl group or in the production of other reactive phenolic compounds from constituents of blood serum as a result of the heat treatment. The former was shown to be negligible by the analysis of known solutions of salicylate before and after the hydrolytic treatment. The latter was excluded by tests on serum blanks which after hydrolytic treatment gave no reaction. A third possible source of error might be the incomplete hydrolysis of the conjugated salicylamide; however, since more prolonged treatment failed to increase recovery, it appears that hydrolysis is complete.

Salicylamide Blood Levels

A group of 24 rabbits were given salicylamide 100 mg./Kg. orally by stomach tube in a suspension. Blood samples were taken one-half to four hours after administration, and salicylamide blood levels determined. To avoid excessive heart punctures not more than three samples were taken from any one rabbit. Another group of 14 rabbits were given salicylamide 300 mg./Kg. orally by stomach tube. Blood levels were determined at one, two, three, and four hours. The results of these analyses are summarized in

Table I. As may be seen, the blood levels reach a peak in one hour and drop rapidly so that after 100 mg./Kg. the blood level is practically zero in three hours. Even after 300 mg./Kg., the blood level falls below 0.5 mg. % in four hours.

TABLE I.—BLOOD SERUM LEVELS OF SALICYLAMIDE

Dose, mg./Kg.	Number of Analyses	Time, Hrs.	Serum Level Mean	Serum Level S. E.
100	6	1/2	5.0	0.68
	16	1	6.9	0.52
	12	2	4.6	0.74
	12	3	0.7	0.18
	6	4	Less than 0.5 mg. %	
300	9	1	23.3	1.90
	5	2	10.8	0.18
	9	3	4.2	1.10
	5	4	0.5	0.32

Repeated Dosage of Salicylamide.—In order to determine whether salicylamide blood levels could be built up and maintained by repeated doses, six rabbits were given 100 mg./Kg. at four-hour intervals five times a day for five days. Blood samples taken daily one hour after the fourth dose showed no increase in the one-hour blood level. Blood samples taken three hours after the fourth dose on the fifth day showed no accumulation of salicylamide. It appears that salicylamide is excreted so readily that blood levels cannot be maintained by doses repeated every four hours.

Doses of 300 mg./Kg. were given to four rabbits every four hours five times a day for two days. Here again analysis of blood samples taken after the fourth dose showed no accumulation of salicylamide.

Absorption and Excretion

To test the rate of absorption and excretion of salicylamide a number of rabbits were given doses of salicylamide of approximately 100 mg./Kg. The drug was administered orally in suspension by stomach tube. The animals were sacrificed after one to four hours. The stomach and intestines were removed and the bladder emptied. The animals had been kept in metabolism cages from time of administration of salicylamide until sacrificed to collect any urine passed in that time. Stomach and intestinal contents were analyzed directly after dilution and thorough mixing with three parts of 1.3 *N* HCl. The results are shown in Table II.

TABLE II.—ABSORPTION OF SALICYLAMIDE FROM GASTROINTESTINAL TRACT

Time, Hrs.	Number of Rabbits	Dose, mg.	Recovery, % Stomach	Recovery, % Intestine
1	3	200	28	0.7
2	4	250	18	1.3
3	4	200	10	1.0
4	5	200	4	...

Urine samples after dilution were hydrolyzed and assayed in the same way as blood samples. The results of these analyses are given in Table III. In collecting twenty-four-hour urine samples, it was not necessary to sacrifice the animals.

It may be seen that the absorption and excretion data harmonize well with the data on blood levels. Rapid absorption gives an early peak blood level, while rapid excretion quickly eliminates the drug and lowers blood levels. Complete recovery of the dose administered was not obtained. However, since twenty-four-hour samples showed only slightly better recovery than four-hour samples, it appears that the drug is almost completely eliminated in four hours or metabolized to some form not detected by the analytical procedure.

TABLE III.—EXCRETION OF SALICYLAMIDE IN THE URINE

Time, Hrs.	Number of Samples	Recovery, % Free Salicylamide	Recovery, % Salicylamide Total
1	3	1.0	33
2	6	8.2	41
3	5	4.9	63
4	5	6.0	81
24	5	9.1	84

Tissue Concentrations.—Attempts were made to recover salicylamide in free and conjugated form from liver, heart, kidney, and striated muscle. These tissues were homogenized in a Waring Blendor with three parts of 1.3 *N*HCl and then treated as in the assay of blood serum. Tissues were removed one, two, and four hours after administration of salicylamide. Liver was found to contain a small amount of free salicylamide one hour after administration. Free salicylamide was not detected in any other tissue. Salicylamide in conjugated form was found in the liver in concentrations of 3 to 10 mg. % after doses of 100 mg./Kg. At that time traces of salicylamide in conjugated form were found in heart and kidney. No salicylamide, free or conjugated, could be found in striated muscle.

Effects of PABA and Ascorbic Acid on Blood Levels.—Since PABA and ascorbic acid have been found to raise the blood levels of salicylate, these drugs were given with salicylamide to determine whether they would be as effective with the latter drug. Nine rabbits were given single doses of salicylamide and PABA, 100 mg. of each per Kg. of body weight. Blood samples were withdrawn from five of these animals one hour and three hours after administration. Blood was taken from the other four animals two and four hours after administration. No appreciable elevation of blood levels was found. In five rabbits given 300 mg./Kg. of salicylamide and PABA, blood samples taken one hour after administration showed a considerable depression of blood levels compared with salicylamide alone. Blood samples taken two and three hours after administration were not significantly different in salicyl content from those after salicylamide.

Ascorbic acid given with salicylamide in doses of 300 mg./Kg. of each had no appreciable effect on blood levels of salicylamide one and three hours after administration.

The results of these experiments are summarized in Table IV.

TABLE IV. BLOOD LEVELS OF SALICYLAMIDE WHEN ADMINISTERED WITH PABA

Time	1 Hr.	2 Hrs.	3 Hrs.
Salicylamide, 100 mg./Kg.	6.9 ± 0.32[a]	4.6 ± 0.74	0.73 ± 0.18
Salicylamide with PABA, 100 mg./Kg.	6.0 ± 0.52	2.8 ± 0.25	0.90 ± 0.78
Salicylamide, 300 mg./Kg.	23.3 ± 1.90	10.8 ± 0.18	4.20 ± 1.10
Salicylamide with PABA, 300 mg./Kg.	11.6 ± 1.60	11.2 ± 0.80	6.00 ± 1.90
Salicylamide with ascorbic acid, 300 mg./Kg.	20.0 ± 0.41	...	5.20 ± 0.96

[a] Figures represent mean blood level ± standard error.

DISCUSSION

From the results of these experiments, it appears that salicylamide is absorbed and eliminated more rapidly than sodium salicylate. Blood levels of free salicylamide from moderately large doses are practically nil. The drug is conjugated very rapidly and almost completely so that it fails to give the ferric-salicyl reaction. The conjugated salicylamide in blood serum can be freed by acid hydrolysis and then analyzed. Whether or not the conjugated salicylamide is pharmacologically significant is still an open question. It is conceivable that free salicylamide is rapidly bound in the tissues, exerting its action there, and that the conjugated salicylamide found in blood serum is a detoxified product released by the tissues and having no pharmacologic action. This problem can, it seems, be solved by preparation of the conjugation products and testing them for pharmacological activity.

The rate of absorption of salicylamide from the gastrointestinal tract of rabbits found in this work agrees rather well with the rate found by Seeberg, *et al.* (4), in the rat. These authors also found blood levels of free salicylamide to be very low after very large doses, a fact confirmed in our work. Total salicylamide as determined in our method, however, is much greater. Seeberg reported 5.6 mg. % in one hour after doses of 500 mg./Kg. as compared with our findings of 23 mg. % in one hour after doses of 300 mg./Kg. In either case the blood levels attained an early peak and then fell rapidly. The fleeting blood levels of salicylamide found in this work on rabbits does not agree with the high prolonged blood levels reported by Euler and Remy (7) in human patients, three and four hours after administration of salicylamide. It may well be that conclusions regarding the rate of absorption and excretion in rabbits cannot be applied to man.

PABA failed to elevate blood levels of salicylamide when given concurrently with that drug. There does appear to be a depression of the peak level of salicylamide but no significant prolongation of the blood levels. This we consider to be due to an initial interference with absorption from the gastrointestinal tract.

SUMMARY

1. A method of analysis of salicylamide is given. This method reveals salicylamide conjugation products after freeing the reactive phenolic hydroxyl group by preliminary acid hydrolysis.

2. Salicylamide blood levels determined by this method were found to reach a peak in one hour, and fall to practically zero in three to four hours after doses of 100 to 300 mg./Kg. in rabbits.

3. Absorption from the gastrointestinal tract was found to be very rapid, 70 per cent in one hour and 90 per cent in three hours. Excretion was also very rapid. We recovered an average of 33 per cent of a dose in the urine in one hour and 80 per cent in four hours.

4. Neither PABA nor ascorbic acid increased the elevation or duration of salicylamide blood levels though PABA did lower the initial peak level of salicylamide given in doses of 300 mg./Kg. to rabbits.

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The Microbiological Determination of Vitamin B_{12} Utilizing a Mutant Strain of *Escherichia coli**

By F. J. BANDELIN and J. V. TUSCHHOFF

A turbidimetric method for the assay of vitamin B_{12} utilizing a mutant strain of *Escherichia coli* is described. The growth response curve is reproducible within satisfactory limits. Methionine interferes only when present in amounts 70,000 times that of the vitamin B_{12}. The recovery of cyanocobalamin added to various liver extracts ranges from 96 to 106 per cent. Determinations carried out on a number of injectable liver and desiccated liver extracts indicate that the *E. coli* mutant method gives results which are comparable to those obtained with the official *Lactobacillus leichmanii* method. The assay of numerous desiccated tissues and liver extracts gave consistent and reproducible results within the normal variation for most microbial methods. Where methionine interference can be avoided, as in the determination of potent vitamin B_{12} concentrates and extracts, this microbial method may have considerable value because of its simplicity and the short time involved in carrying out determinations.

THE MICROBIOLOGICAL determination of vitamin B_{12} in natural-occurring materials and their concentrates is generally accomplished using two strains of *Lactobacillus leichmanii*, ATCC 4979 and ATCC 7830. The latter constitutes the test organism of the official U. S. P. method for the determination of vitamin B_{12}. The complex growth requirements and lack of specificity of these organisms make vitamin B_{12} assays with them time-consuming and variable.

The possibility of a simplified procedure utilizing a less fastidious organism, without sacrificing specificity or sensitivity, is worthy of investigation for the assay of the vitamin B_{12} activity of certain natural products commonly encountered in pharmaceutical preparations.

Although Euglena responds to vitamin B_{12} more specifically and at a much lower concentration than does *L. leichmanii*, the four- to seven-day incubation period required for it is a distinct deterrent to its use in routine assay work.

In 1950 Davis and Mingioli (1) reported the isolation of a number of mutant strains of *Escherichia coli* produced by selection from ultraviolet irradiated cultures of *E. coli* (ATCC 9637). One strain, 113-3 showed marked responses to both methionine and vitamin B_{12} as compared with the requirements for much higher amounts of methionine. These investigators observed that the growth of this *E. coli* mutant, in the absence of vitamin B_{12}, could not be satisfied by a variety of natural-occurring compounds including certain amino acids, vitamins of the B-complex, purines, pyrimidines, ribonucleotides, ribonucleosides, thymidine, and intermediates of the vitamin B_{12} molecule.

In 1951 Burkholder (2) reported on the turbidimetric determination of vitamin B_{12} using the *E. coli* mutant 113-3 and a basal medium after the formula employed by Davis and Mingioli with additional organic sources of nitrogen in accordance with results of tests made with supplements of single amino acids.

Because of the relatively simple synthetic medium required, ease of growing and propagating the organism and specificity for vitamin B_{12}, we undertook to determine the applicability of the *E. coli* mutant 113-3 as an assay organism for various pharmaceutical materials. We have used Burkholder's medium with certain modifications in technique and handling for a tur-bidimetric method for the assay of vitamin B_{12}-containing products and for comparison with the official *L. leichmannii* method.

EXPERIMENTAL

The basal medium used for the determinations made double strength is as follows:

Potassium phosphate, dibasic	7.0
Potassium phosphate, monobasic	3.0
Sodium citrate, dihydrate	0.5
Magnesium sulfate, heptahydrate	0.1
Ammonium sulfate	1.0
Dextrose	10.0
Asparagine	4.0
Arginine	0.1
Glutamic acid	0.1
Glycine	0.1
Histidine	0.1
Proline	0.1
Tryptophan	0.1
Sodium thioglycollate	0.1
Water	500

All ingredients were dissolved in the water by shaking on a shaking machine or by stirring with a mechanical stirrer. The *p*H was adjusted to 6.8. Five milliliters of the double strength medium was added to each tube containing a solution of standard or unknown sample and sufficient water was added, where necessary, to make the total volume exactly 10 ml. The tubes were plugged with cotton plugs or covered with aluminum caps and sterilized by

* Received November 13, 1953, from the Research Laboratories of Flint, Eaton and Co., Decatur, Ill.

autoclaving for five minutes at 110°, then cooled rapidly by immersing in cold water. Autoclaving at higher temperatures, prolonged heating, or failure to cool tubes immediately after removing from the autoclave will cause discoloration of the medium. Incorporation of the thioglycollate appears to protect the vitamin B_{12} from destruction in the presence of natural materials during autoclaving (3, 4).

Following rapid cooling in water, each tube is inoculated with a drop of freshly prepared suspension of *E. coli* mutant. Stock cultures of the assay organism are carried on Difco® nutrient agar. The inoculum is grown in Difco® nutrient broth for about ten hours prior to use. The organisms are prepared for use by washing with saline, centrifuging, and resuspending in isotonic saline solution in the usual fashion. After inoculation of the test medium it was found that adequate growth could be obtained after fifteen to eighteen hours of incubation at 32°. Growth is determined by measuring the turbidity of the medium in a Coleman spectrophotometer or other suitable spectrophotometer at 650 mμ with no filter.

Cultures of the *E. coli* mutant were carried on nutrient agar slants stored in a refrigerator after incubating at 32° for sixteen to twenty-four hours. Inoculum was prepared by taking the organisms from the slant and growing them in ordinary broth. Perceptible growth in the broth in four hours or less was used as the criterion of adequate growth rate of the organisms for the assay. If growth did not develop before four hours the organisms were repeatedly transferred to fresh nutrient broth on consecutive days until the growth rate accelerated to a point at which visible growth occurred between three and four hours after transfer. From a six-hour culture meeting these growth requirements, inoculum was prepared as previously described.

RESULTS

A typical growth response to crystalline vitamin B_{12}[1] is given in Fig. 1 showing growth response plotted on semilog paper with the log response plotted against the arithmetic dose. The half maximum growth level occurs at about 0.3 mμg. of B_{12} per milliliter. It was found that adequate and consistent growth could be obtained without constant shaking as recommended by Burkholder. In avoiding aeration by shaking the tubes during incubation the growth responses became smaller with the resulting smaller assay range. Reproducibility of the standard curve is illustrated in Table I. Under specified conditions of the assay, the organism responds similarly to vitamins B_{12} and B_{12a} (5).

The vitamin B_{12} content of some commercial liver preparations was determined by the *E. coli* mutant and the official *L. leichmanii* method.[2] Various samples of desiccated liver, N. F., liver injection, U. S. P., and several liver fractions were assayed by both methods. The desiccated liver and the solid liver fractions were extracted by heating with water on a steam bath for thirty minutes, then filtering and diluting with water to give a final solution containing approximately 0.2 mμg./ml. Results are given in Table II.

[1] Cyanocobalamin (Merck) U. S. P.
[2] Difco® Vitamin B_{12} assay media U. S. P. dehydrated was used in these determinations.

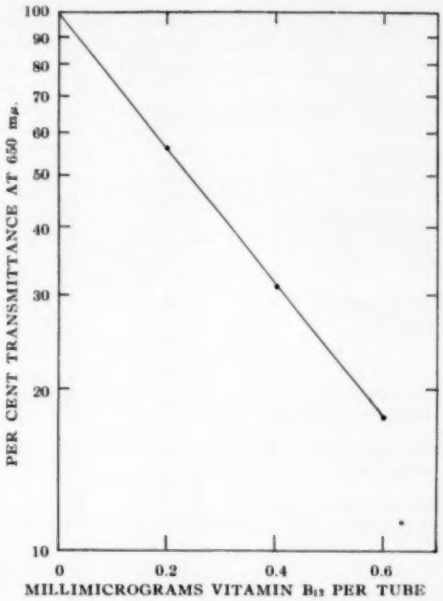

Fig. 1.—Growth response to crystalline vitamin B_{12}.

A number of liver extracts including injectable liver extracts as well as aqueous extracts of solid liver preparations were assayed by the *E. coli* method, then measured increments of crystalline cyanocobalamin were added to each tube, and the assays run to determine the total B_{12} content and to check the recovery of vitamin B_{12}. Results are given in Table III.

Finally, a number of determinations on some pharmaceutical preparations were carried out in order to determine the reproducibility which might be expected under actual conditions of use. Table IV lists the vitamin B_{12} content of various desiccated tissues and extracts used in the pharmaceutical industry as determined by the *E. coli* mutant method.

DISCUSSION

Since the mutant 113-3 requires either vitamin B_{12} or methionine for growth in the medium, the possibility of methionine interference in B_{12}-containing materials, especially liver preparations, was investi-

TABLE I.—REPRODUCIBILITY OF THE STANDARD CURVE

Cyanocobalamin	Colorimetric Reading at 650 mμ: Range for 20 Determinations	Mean of Variation
0.0	97–100	99.1
0.1	74–77	75.8
0.2	53–61	56.1
0.3	38–44	41.0
0.4	27–35	31.0
0.5	20–27	51.4
0.6	15–21	18.3

TABLE II.—VITAMIN B_{12} CONTENT OF LIVER EXTRACTS ASSAYED BY E. COLI AND L. LEICHMANII

Sample	*L. leichmanii*, μg. B_{12}/Gm.	*E. coli*, μg. B_{12}/Gm.
Desiccated hog liver N. F. (*A*)	0.475	0.568
	0.700	0.524
	0.520	0.538
Desiccated hog liver N. F. (*B*)	0.520	0.550
	0.625	0.520
	0.530	0.500
Desiccated and defatted hog liver N. F. (*C*)	0.625	0.650
	0.800	0.625
	0.650	0.650
	μg. B_{12}/ml.	μg. B_{12}/ml.
Liver injection U. S. P. 2 μg./ml.	1.60	1.80
	1.60	1.80
	2.40	2.50
	2.35	2.60
	2.20	2.35
Liver injection U. S. P. 10 μg./ml. aged sample	7.5	8.8
	7.0	9.2
Liver injection U. S. P. 10 μg./ml.	12.0	9.6
	13.0	12.2
	10.5	12.6
Liver injection U. S. P. 20 μg./ml.	22.0	18.0
	26.5	20.2
	18.4	20.8
	19.0	20.6
	23.6	21.6

gated. For testing the interference of methionine, increasing increments of L-methionine were added to tubes containing graded amounts of vitamin B_{12}. The recovery of vitamin B_{12} was determined. Burkholder has reported that the half maximum growth of the mutant occurs at a level of approximately 6 mμg./ml. or about 50,000 times the concentration of vitamin B_{12} required to produce an equivalent amount of growth; our figures for the present method indicate that the ratio may be nearer to 70,000 times the amount of methionine required to produce an equivalent growth response of the mutant. Since the methionine content of commercial liver preparations and liver extracts is far below this limiting ratio, methionine does not ordinarily interfere in the method.

TABLE III.—RECOVERY OF ADDED CYANOCOBALAMIN

Sample	Apparent B_{12} Content/Tube, μg.	Cyanocobalamin Added/Tube, μg.	Total B_{12} Activity Found, μg.	Cyanocobalamin Recovered, Per Cent
Liver ext.:				
No. 1	0.31	0.20	0.52	102
No. 2	0.21	0.20	0.42	106
No. 3	0.22	0.20	0.44	104
No. 4	0.25	0.20	0.43	96
No. 5	0.21	0.20	0.43	105
No. 6	0.15	0.40	0.56	102
No. 7	0.18	0.40	0.46	96
Liver prepn.:				
No. 4	0.28	0.20	0.48	100
No. 7	0.31	0.20	0.52	102
No. 8	0.11	0.40	0.54	106

The extraction procedure for desiccated tissues was standardized as follows: 1–5 Gm. of the solid were extracted with 100 ml. of water by heating to 60° on a water bath for one hour. The resulting solution was filtered through paper and the filtrate adjusted to volume. Serial dilutions were made to arrive at a concentration of vitamin B_{12} activity within the assay range (0.1–0.2 mμg./ml.) or in not more than the 5 ml. required to dilute the media. The use of such aqueous extracts gave fairly consistent and reproducible results.

The *E. coli* mutant 113-3 seemed to give slightly higher results than did the *L. leichmanii* but well within the limitations of the official method. Results obtained by the two methods generally are in good agreement.

TABLE IV.—VITAMIN B_{12} CONTENT OR ACTIVITY OF VARIOUS DESICCATED TISSUES OR EXTRACTS BY E. COLI MUTANT METHOD

Substance	B_{12} Content Determinations on Same Sample, μg./Gm.
Hog stomach desiccated	0.05
	0.06
	0.06
	0.06
	0.05
Duodenum desiccated	0.10
	0.08
	0.09
	0.10
	0.09
Liver desiccated equine	1.25
	1.20
	1.28
	1.35
	1.30
Liver fraction 1, N. F.	1.32
	1.45
	1.30
Liver injection, U. S. P., 20 μg. B_{12} activity per cc.	16.50
	17.00
	17.00
	17.55
	16.00
Liver injection, U. S. P., 20 μg. B_{12} activity per cc.	22.40
	26.20
	24.25
	22.00
	22.75
Liver injection, U. S. P., 2 μg. B_{12} activity per cc.	2.45
	2.35
	2.40
	2.35
	2.20
Liver desiccated and defatted, N. F.	0.64
	0.62
	0.64
	0.65
	0.65

SUMMARY

A turbidimetric method for the microbiological determination of vitamin B_{12} utilizing the *E. coli* mutant 113-3 gives results with cyanocobalamin standards and with various biological tissues and extracts which are comparable to those obtained by the official *L. leichmanii* method.

There is considerable difference in the growth response curves between tubes shaken during incubation and tubes not shaken. Tubes not shaken show a much steeper growth response curve reaching maximum growth in a shorter period of time.

Methionine interferes with the determination if present in concentrations of more than 70,000 times that of vitamin B_{12}.

Results of a number of determinations on various desiccated biological tissues and extracts indicate that the method is reproducible and gives consistent results within the inherent variation of similar microbiological procedures.

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A Simple Method for the Determination of Vapor Pressure of Volatile Amines*

By FRANK A. STELDT and K. K. CHEN

A simple method has been designed for the estimation of volatile amines which is particularly useful for the assay of the inhalers of vasoconstrictors.

IN THE COURSE of an investigation of volatile amines it became desirable to determine the vapor pressures of amino compounds which were volatile vasoconstrictors, and to extend the method to an evaluation of inhalers containing medication that included these amines in varying quantities.

EXPERIMENTAL

The method selected as being most useful was an adaptation of Walker's method (1). The vapor pressure was calculated from the amount of the material required to saturate a known volume of dry, carbon dioxide-free air. In extending the work to inhalers, the amount of material evaporated per

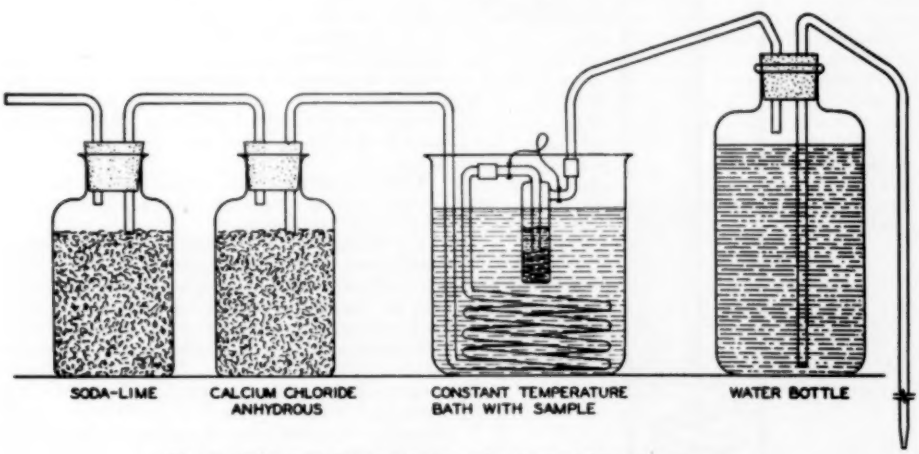

Fig. 1.—Diagram of apparatus used for determining vapor pressure.

* Received November 27, 1953, from the Lilly Research Laboratories, Indianapolis 6, Ind.

TABLE I.—TEST RUNS WITH ETHANOL AND THREE AMINES

Compound	Run	Temperature, °C.	Amount Evaporated, Gm.	Vapor[a] Pressure, mm. Hg	Average Vapor Pressure, mm. Hg
Ethanol	1	26	0.6590	65.64	
	2	26	0.6157	61.36	63.49
β-Phenethylamine	1	25	0.0091	0.346	
	2	25	0.0078	0.296	0.312
	3	25	0.0077	0.293	
1-Phenyl-2-aminopropane	1	26	0.0052	0.179	
	2	26	0.0084	0.292	0.256
	3	26	0.0086	0.296	
2-Aminoheptane	1	26	0.1453	5.871	
	2	26	0.1435	5.798	5.812
	3	26	0.1427	5.766	

[a] Atmospheric pressure was 739.5 mm.

known volume of air was calculated instead of the vapor pressure, since this figure had a more practical bearing on the problem. The same principle has been applied to the determination of the vapor pressure of iodine (2).

Figure 1 shows a diagram of the apparatus used for determining vapor pressure. A large bottle of known volume is filled with water, which is siphoned out to provide the "motive power" to draw a stream of air of known volume through the apparatus. The air passes through soda-lime, anhydrous calcium chloride, and a constant temperature bath before entering the evaporator. Figure 2 shows the construction of the evaporator. A glass spiral surrounds the inlet tube to provide a longer path for the air bubbles.

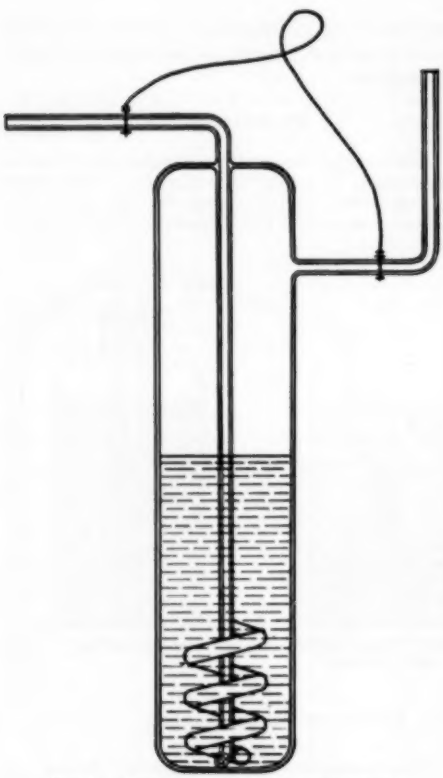

Fig. 2.—Construction of the evaporator.

In operation, the evaporator was loaded with 3–4 cc. of the liquid under investigation, wiped with a clean, dry towel, and weighed. It was placed in the bath and connected to the system, the siphon started, and the air allowed to pass through until the bottle was empty. In all of these experiments a bottle of 4-L. capacity was used. The time required to saturate this volume of air was about ten minutes as found by experiment. Slower rates of flow had no influence on the results. The outflow from the bottle was therefore regulated to provide for emptying the bottle of water in about ten minutes, and this adjustment was kept constant. After the air had passed through, the evaporator was removed, wiped carefully with a clean, dry towel, and weighed. The loss in weight was determined and substituted into the formula:

$$p = \frac{g}{mv} RT$$

wherein p is the vapor pressure in atmospheres, R is the molar gas constant in liter-atmospheres, T is the absolute temperature, g is the observed loss of weight in grams, m is the molecular weight of the liquid used, and v is the volume of air used in liters. Multiplying p by the barometric pressure at the time of observation gives the result in millimeters of mercury.

Table I gives the results of observations on three volatile amines. Absolute ethanol was used as a check on the apparatus.

The method used to evaluate the behavior of medicament-containing inhalers was slightly different. Figure 3 is a photograph of apparatus in actual use, modified to provide a holder for the inhaler itself in place of the evaporator; an absorption tube containing aqueous acid is introduced between the inhaler and the water bottle. The method of operation was similar, in that the inhaler was placed in the holder and a known volume of air allowed to pass through. However, the air saturated with amine vapors from the medicament was led through the absorption tube, in which a known volume of standard acid had been placed, and the amine was absorbed in the acid. The air and nonbasic vapors passed out through the bottle. Suitable precautions

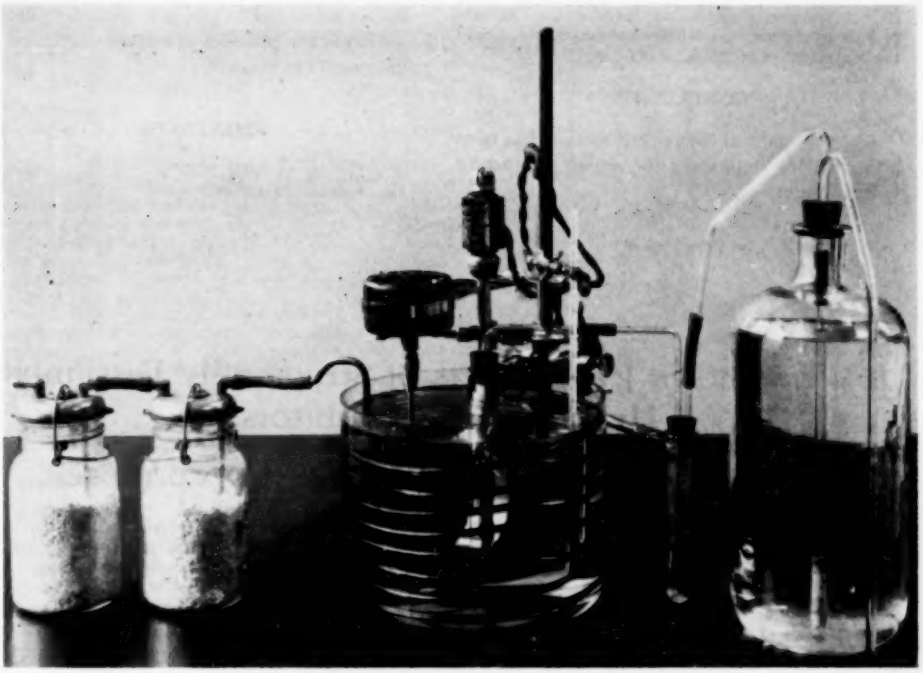

Fig. 3.—Photograph of apparatus used.

TABLE II.—ASSAY OF AN INHALER OF 2-AMINO-4-METHYLHEXANE P-39993-J

Run	mg. Base/4 Liters of Air
1	5.96
2	5.96
3	5.90
4	5.68
5	5.39
6	5.42
7	5.39
8	5.14
9	5.00
10	4.97
11	4.87
12	4.79

were taken to insure recovery of any acid in the connecting tubes and on the stopper of the absorption tube. The free acid remaining in the absorber was then titrated with a standard solution of sodium hydroxide using bromothymol blue as the indicator. The amount of amine evaporated was calculated by the formula:

$$mg \text{ base} = D \cdot m \cdot ME$$

where D is the difference between the cc. of base equivalent to the acid initially in the absorber, and the cc. of base equivalent to the acid remaining after absorption of the amine vapors; m is molecular weight of the amine, and ME is milliequivalents of base per cc. Blank runs resulted in a consumption of less than 0.01 ME of acid, which can be disregarded for all practical purposes, as it is substantially constant.

By making successive runs on the same inhaler and plotting on a graph mg. of amine base volatilized against liters of air used, a curve can be obtained which is characteristic of the preparation in the inhaler. A flat curve with little or no slope is desirable and indicates a fairly constant vaporization of amine from the medicament in the inhaler.

Table II gives the results from the examination of a typical inhaler containing 2-amino-4-methylhexane dissolved in a vehicle which suppresses the volatility of that amine. The mixture is absorbed on a cotton plug. Figure 4 is the graph obtained from these results. In this experiment 2 cc. of 0.1 N HCl was placed in the absorbing tube before each run. This was back-titrated with 0.025 N NaOH solution,

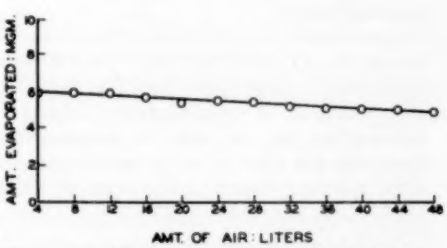

Fig. 4.—Results from examination of typical inhaler.

freshly prepared before the experiment. The rate of flow of the air was 4 liters in nine minutes, and the temperature of the air was maintained at 25°.

CONCLUSION

1. A simple and convenient method has been presented for estimating the vapor pressure of volatile amines.

2. This method has been extended to determination of the behavior of amines when used as medicaments in inhalers.

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In Vivo and *in Vitro* Effects of Structurally Dissimilar Hyaluronidase Inhibitors*

By J. M. BEILER, R. BRENDEL, and GUSTAV J. MARTIN

Hyaluronidase inhibitors of three general structural types have been tested both *in vitro* and *in vivo*. *In vivo* tests were designed to demonstrate alterations in permeability associated with the following: (*a*) subcutaneous spreading, (*b*) absorption from a subcutaneous or intramuscular injection site, (*c*) experimentally induced inflammation, and (*d*) experimentally induced capillary trauma.

FOR A NUMBER OF YEARS a research program devoted to the investigation of hyaluronidase and hyaluronidase inhibitors has been in progress in this laboratory, based on the assumption that factors capable of modifying tissue and cell permeability would assume increasing therapeutic importance. Recent findings have reinforced this view. For example, it has been suggested (1, 2) that the inflammatory process is associated with hyaluronidase imbalance, and that the beneficial effect of compounds of the cortisone type in conditions characterized by inflammation is due to their ability to inhibit hyaluronidase. The knowledge that hyaluronidase facilitates the absorption of fluids and drugs when administered by hypodermoclysis (3) suggested that hyaluronidase inhibitors would have a general therapeutic application; that is, that by slowing absorption from a site of injection and creating a depot effect they would prove useful as adjuvants in parenteral administration of medicinal substances.

Investigation has shown that phosphorylated hesperidin, a specific inhibitor of hyaluronidase both *in vitro* (4) and *in vivo* (5), will in fact inhibit the formation of experimentally induced inflammations (6). In addition, phosphorylated hesperidin has been shown in the laboratory to delay the absorption of intramuscularly administered heparin (7) and trypsin (6) and to increase the efficiency of immunization reactions (8) by virtue of its depot-forming properties. Reports of similar actions by other hyaluronidase inhibitors have recently appeared. Polyphloretin phosphate (9) has been shown to be a desirable adjuvant in ACTH administration, at least in part because of its ability to delay the absorption of the hormone from the site of injection. Polymers of diphenylmethane and triphenylmethane (10) have been reported to inhibit development of egg-white edema in the laboratory; some of these compounds (11) have been tested clinically with favorable results.

Tests run in this laboratory with compounds structurally related to phosphorylated hesperidin (5) have shown that *in vitro* antihyaluronidase activity may be found without corresponding activity in the body. The reports (9, 10) of *in vivo* antihyaluronidase activity by substances dissimilar in structure to each other and to phosphorylated hesperidin suggested that an investigation of the comparative *in vitro* and *in vivo* activities, by several different testing procedures, of the three types of compound would be of value.

EXPERIMENTAL

The reported compounds were synthesized in this laboratory by the usual methods except for one sample of polyphloretin phosphate and one of trigentisic acid which were obtained from outside

* Received November 24, 1953, from the Research Laboratories, The National Drug Co., Philadelphia, Pa.

TABLE I.—EFFECT OF HYALURONIDASE INHIBITORS IN VITRO AND IN VIVO

Compound	*In Vitro* Hyaluronidase Inhibition MEC (mg./ml.)	Subcutaneous Dye Spread Inhibition —Hyaluronidase— With	Without	Egg-white Edema Inhibition, Per Cent	Capillary Permeability (Time over Control)
Trigentisic acid	0.001	+	0	30	..
Polytrigentisic acid 1307	0.001	+	+	0	..
Polytrigentisic acid 1308	0.001	+	0	25	..
Polytrigentisic acid 1309	0.001	+	+	0	..
Polydicarboxytetrahydrodiphenylmethane	0.0001	+	0	30	..
Polytricarboxyhexahydrotriphenylmethane	0.001	+	0	0	1
Polygallic acid phosphate	Negative	0	0	0	1
Polyphloroglucinol phosphate	0.001	+	+	30	2
Polyphloretin phosphate	0.01	+	0	15	2
Polycondensed hesperetin	Negative	0	0	0	..
Polyhesperetin phosphate	0.01	+	0	25	2
Poly-2′,4-dihydroxy-5′-carboxychalcone	0.001	0	0	0	..
Phosphorylated 2′4-dihydroxy-5′-carboxy-chalcone	0.01	0	0	0	..
Phosphorylated-poly-2′,4-dihydroxy-5′-carboxychalcone	0.01	+	0	0 45	..
Phosphorylated hesperidin	0.001	+	+	25 (i. p.)	5
Trigentisic acid (o)	0.001	0	0	0 (i. p.)	1
Polyphloretin phosphate (o)	0.0001	+	+	10 (i. p.)	2

sources. The phosphorylated hesperidin used throughout was lot A-25, the product of a routine pilot plant run.

The *in vitro* hyaluronidase assay was done turbidimetrically by the method of Seastone (12) as previously reported (13). The hyaluronic acid used was prepared from human umbilical cord and the hyaluronidase was a commercial sample from bovine testes.

The dye-spreading experiments were carried out by a modification of the method of Mayer and Kull (14). Adult rats were depilated on both flanks. Each animal received on one flank a subcutaneous injection of a mixture of 0.05 ml. of India ink and 0.1 ml. of saline or a hyaluronidase solution containing 100 T.R.U. After thirty minutes the area of spread was traced on cellophane paper. The animal was then given the compound being tested by intraperitoneal injection. Thirty minutes later the administration of India ink with or without hyaluronidase was repeated on the other flank and the area of spread determined. Decrease in area of spread following injection of the compounds being tested was taken as indicating an inhibition of the spreading action of hyaluronidase. All compounds were given at a level of 100 mg./Kg.

Effects on capillary permeability were determined by the method of Ambrose and DeEds (15). Twenty-four hours before the test the abdominal area of rabbits was depilated. On the day of the test the animals were given 2 ml. of a 1% trypan blue solution by marginal ear vein injection. Five minutes later chloroform was applied to the skin of the abdomen for thirty seconds by means of a cotton applicator. The time required for appearance of the blue dye at the site of chloroform application was recorded. Following a number of control determinations the compound being tested was administered intravenously. After five minutes chloroform was again applied and the time required for appearance of the dye determined. An increase in time of appearance over the control was interpreted as indicating an inhibitory effect on the chloro orm-induced increase in capillary permeability. All compounds were administered at a dosage of 100 mg./Kg.

Anti-inflammatory effects were determined by assaying the inhibition of the development of egg-white edema (10). Rats weighing 60–90 Gm. were used. Compounds were administered orally at 200 mg./Kg. for the most part. In a few cases compounds were given i. p. in two doses of 20 mg./Kg. each, thirty minutes apart. Immediately after the second i. p. dose, or thirty to sixty minutes after the oral dose, 0.1 ml. of egg-white was injected subcutaneously into the dorsal side of one hind paw and 0.1 ml. of saline into the other. After ninety minutes the animals were killed with ether. Both hind legs were removed at the knee joint and weighed. The difference in weight between the saline-injected and egg-white-injected legs was taken as indicating the extent of edema. A decrease in weight gain in the edematous leg over that of controls run without inhibitors was taken as indicating an inhibition of edema formation.

ACTH assays were carried out on hypophysectomized male Sprague-Dawley rats. One U. S. P. unit of purified corticotropin in 0.2 ml. of 15% gelatin was injected subcutaneously. Compounds whose effect was tested were made up in 4% solution in the gelatin-corticotropin mixture. Two groups of animals were run with each compound; in each case, the left adrenal was removed immediately prior to the injection and initial ascorbic acid levels determined. In one group the right adrenal was removed three hours, in the other six hours after the injection. Ascorbic acid levels were determined by the method of Mindlin and Butler (16).

Effects on heparin absorption were determined on rats. Heparin was given by intramuscular injection at a dosage of 2,000 units/Kg. in 0.5 ml. of saline. Compounds tested were incorporated into the heparin solution at the 4% level. Cardiac blood samples were withdrawn from the animals at zero time and at intervals thereafter; 0.8 cc. of blood was introduced into a test tube of 9 mm. diameter and the time at which the blood ceased to flow on inversion of the tube was taken as the clotting time.

RESULTS AND DISCUSSION

In Table I are presented results on some of the tests routinely used in this laboratory to assay *in vivo* antihyaluronidase activity. Figures on *in vivo* results represent in every case the average values obtained with groups of five animals. The results on subcutaneous dye spreading are reported as positive or negative because of the difficulty in obtaining accurate measurement of the irregularly shaped areas of spread. The results on the egg-white edema test were obtained throughout with oral dosage except where this is noted as being i. p. The figures obtained in the capillary permeability test are given in length of time for appearance of the dye over the control time; the latter, of course, varied somewhat in individual animals but averaged about two minutes. All of the compounds tested were prepared in this laboratory except the two designated (0), which were obtained from outside sources.

It would appear, first, that there is some definite correspondence between the *in vitro* and the *in vivo* results, inasmuch as those compounds which were inactive *in vitro* gave negative results in all the *in vivo* tests. The reverse does not hold true: two of the three chalcones tested as well as the sample of trigentisic acid obtained from outside sources gave completely negative *in vivo* results, despite the fact that all showed definite *in vitro* antihyaluronidase activity.

The third chalcone derivative tested gave positive *in vivo* results only in the subcutaneous dye-spreading test with added hyaluronidase. This test would appear to be in a sense nonphysiological, since it depends on administration of exogenous hyaluronidase and therefore cannot be considered to be a measure of actual hyaluronidase activity in the body. The test run without added hyaluronidase is much more specific; unfortunately, the small areas of spread require that a large inhibitory effect be obtained for significance.

Of the compounds tested, only three—polyphloroglucinol phosphate, phosphorylated hesperidin, and the sample of polyphoretin phosphate obtained from outside sources—gave positive results in all the *in vivo* tests. Factors such as rate of absorption, breakdown, and excretion, as well as ability to combine with the enzyme involved at different sites in the body, may account for the variation of results in the different tests.

It is of interest to note that small variations in the method of preparation can cause profound differences in physiological activity. The trigentisic acid prepared in this laboratory showed some physiological activity, while that obtained from outside sources was completely inactive *in vivo;* three samples of polytrigentisic acid prepared here gave quite different *in vivo* results. This influence of the mode of preparation on physiological activity has also been noted with phosphorylated hesperidin (5).

Tables II and III show the effects of the three types of hyaluronidase inhibitors tested on the absorption of ACTH and heparin, respectively. All figures are averages for groups of five animals. In these tests the polyphoretin phosphate and trigentisic acid samples used were those obtained from outside sources. The trigentisic acid, which was without activity in the *in vivo* tests reported in Table I, was also essentially inactive in the absorption tests. Phosphorylated hesperidin was superior to polyphloretin phosphate in delaying the absorption of heparin; with ACTH the results were identical. It was noted in preparing the polyphloretin phosphate–ACTH solution for injection that a precipitate formed. The depot action of the polyphloretin phosphate in this case may therefore be due in part to the formation of an insoluble complex with the ACTH.

TABLE II.—EFFECT OF HYALURONIDASE INHIBITORS ON ACTH-INDUCED ASCORBIC ACID DEPLETION

Compound Injected	Drop in Adrenal Ascorbic Acid (mg./100 Gm. Adrenal Gland) 3 Hrs.	6 Hrs.
Control	96	47
Trigentisic acid	104	80
Polyphloretin phosphate	68	100
Phosphorylated hesperidin	51	95

TABLE III.—EFFECT OF HYALURONIDASE INHIBITORS ON ABSORPTION OF HEPARIN

Time (Min.)	Clotting Time— Control	Phosphorylated Hesperidin	Polyphloretin Phosphate	Trigentisic Acid
0	1 min. 55 sec.	1 min. 47 sec.	1 min. 55 sec.	2 min. 16 sec.
30	No clot	18 min.	14 min.	No clot
60	No clot	4 min. 18 sec.	No clot	No clot
120	No clot	No clot	No clot	No clot
180	No clot	No clot	No clot	No clot

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The Use of Polyethylene Glycol in Tablet Coating*

By EUGENE H. GANS and LEONARD CHAVKIN†

An improved method for the coating of medicinal tablets using polyethylene glycol 6000 has been developed as a result of this investigation. Using conventional tablet coating equipment but none of the conventional materials, this process is offered as a replacement for the usual sugar coatings over which it possesses many distinct advantages. With this new process, tablet coatings have been produced which are stable, durable, readily water-soluble, and evenly colored. The time required for the application of this coating is less than half that currently spent in sugar coating and since no water is used in the coating solutions, the need for protective coatings is eliminated. The polyethylene glycol used is nonhygroscopic, noncaloric, and relatively nontoxic. Its solubility in a variety of common solvents makes the new process very flexible.

THE PURPOSE of the investigation reported in this paper was to devise an improved method or establish a refinement of the present method for the coating of medicinal tablets. The investigation was concerned with the sugar coating generally applied to tablets for purposes of imparting elegance or distinction to the product, and not with protective or enteric coatings which, if needed, are applied prior to the sugar coating.

Current tablet-coating procedure is an old and somewhat mystical art and is somewhat superficially treated in the standard pharmaceutical reference works. There has been slight attention paid to the problems of tablet coating by recent investigators, and as a result, the techniques involved have undergone little change or refinement since they were first developed.

Tablets are sugar coated for several reasons, but basically these are concerned with the elegance and distinction the coating confers upon the product. The process that is currently employed is a long and rather tedious one. In addition, it requires considerable skill and experience on the part of the operator to achieve a satisfactory product. With even the most experienced personnel the process requires approximately three days for completion, depending upon the size, shape, and nature of the tablet as well as the intensity of the color to be applied.

Many attempts have been made to shorten the process by investigators at Columbia University College of Pharmacy prior to this study. These attempts were concerned with modifications of the usual tablet-coating techniques with the use of conventional tablet-coating materials. The results obtained by using varying concentrations of syrup and color, different temperatures, different speeds of rotation, and changing some of the other variables involved, indicated that no appreciable reduction in coating time could be accomplished by these means.

It became apparent that any major reduction in coating time might be effected only if the basic practices in tablet coating were revised. This meant that either the equipment or the materials employed must be altered radically.

A critical examination of the materials utilized in the current coating process revealed one outstanding feature which was believed to be the major cause of the length of the process. Sucrose is the basic material used and it is not plastic. It is a hard, brittle, crystalline substance which powders upon abrasion. This is a serious drawback to the efficient coating of tablets since the smoothing process is prolonged by the fact that sucrose coatings become roughened when the tablets are tumbled in the coating pan. If a material could be selected which would be plastic enough, even when dry, to spread over the tablets without becoming roughened, the tablets would be easier to coat uniformly and elegantly.

In addition to this basic factor, there are several criteria which a material to be used in tablet coating must meet. They are:

(*a*) The material must be relatively nontoxic. Since sizes of tablets and their frequency of ingestion vary over a wide range, the material must have a low acute and chronic toxicity.

(*b*) It should be white or colorless so as to allow the use of the wide variety of colors common to coated tablets.

(*c*) It should be a solid, stable to the effects of air, light, and heat and humidity with which tablets may come into contact.

(*d*) It should be easily applied to the tablets without requiring unusual apparatus or involved techniques.

(*e*) It should be chemically nonreactive to preclude incompatibility with any of the medicaments used in tablets.

* Received December 30, 1953, from the College of Pharmacy, Columbia University, New York, N. Y.
Presented to the Pharmacy Subsection, A. A. A. S., Boston meeting, December, 1953.
Abstracted from a thesis presented to the Graduate Division of Columbia University College of Pharmacy by Eugene H. Gans in partial fulfillment of the requirements for the degree of Master of Science.
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(*f*) It should be rapidly soluble in the fluids of the gastrointestinal tract.

(*g*) It should be soluble in volatile solvents such as ethanol, to reduce the drying time of the coats and to prevent attack upon water-soluble components or the disintegrating agents commonly found in tablets.

(*h*) The cost of the material should be low enough so that its use could be economically feasible.

(*i*) It should be practically odorless and tasteless.

An examination of the available materials in the light of these criteria led to the selection of Carbowax ® 6000[1] for this investigation since it appeared to satisfy all of the requirements.

Although more expensive than sucrose, its cost in bulk quantities is low enough to permit its use as a tablet-coating material. This is especially true since the high labor cost of the tablet-coating process makes the cost of the materials appear rather negligible.

The toxicity of Carbowax ® 6000 appears to be low enough to permit its use in this application. Toxicity studies have indicated that the material is poorly absorbed and that modern production methods have resulted in compounds of extremely low toxicity (1–5). A review of the properties and methods of analysis of polyethylene glycols can be found in the volume by Curme and Johnston (6).

EXPERIMENTAL

The uncoated tablets used in these experiments were composed of terra alba, granulated with acacia, 10% aqueous solution. They were compressed on a Stokes model B-2 rotary tablet machine using $^{13}/_{32}$-inch diameter punches and weighed 0.4 Gm. each. Both standard and deep concave punches were employed to discover whether the coating procedure would be equally successful with both shapes.

The Carbowax® 6000 used was the usual commercially available material and was not treated in any manner prior to dissolving it in the appropriate solvent.

The colors employed were the typical Food and Drug Administration certified colors commonly used to color medicinals. The concentrations of color referred to are based upon the dry weight of the Carbowax®.

The coating was carried out with laboratory size, bench type, copper coating pans. Blowers were available for supplying heated air to aid in drying and exhaust pipes were present to remove accumulated powder. A canvas-lined pan was used for polishing the finished tablets.

First attempts were directed at determining whether it would be possible to apply an adherent film of Carbowax® which would be hard enough, yet not chip off the tablets. It was found that the size of the particles of Carbowax® deposited on the tablets determined the adhesiveness of the film. Thus the concentration and rate of drying of the first Carbowax® coats are crucial factors which influence the quality of the base coat.

A warm 25% alcoholic solution of Carbowax,® applied so as to just cover the tablets, deposited a thin, translucent, adhesive film which did not chip or crack off. The use of higher concentration resulted in coatings which were uneven and did not adhere as well.

In building up the bulk of the tablet coat, a warm 50% alcoholic solution of Carbowax® was found to give the best results. A smooth coat was rapidly deposited without any operational difficulties. Increased concentrations resulted in unevenness and sticking together of the tablets.

Color was applied to the tablets by dissolving the color in an alcoholic solution of the Carbowax®. The use of a warm 40% colored alcoholic solution resulted in the deposition of a smooth, evenly distributed color coat. The concentration of color could be varied from 0.1 through 0.25% (based upon the weight of Carbowax®) depending upon the intensity of the color desired. If a greater concentration of color is used, it will be easily transferred when handling the finished tablets. In contrast to what is usual in sugar coating, it was found that dark colors were as easy to apply as the lighter shades.

The finished coat, although uniform and smooth, was found to be easily scraped off the tablet. This difficulty was eliminated by placing the tablets in an oven at 50–55° for three or more hours and subsequently allowing the tablets to cool to room temperature. After this treatment the tablet coat was adhesive and hard, yet not brittle.

Tablets compressed by standard concave punches were as successfully coated as those made by deep concave punches although they required a slightly longer coating time.

A series of materials were added successively to the Carbowax®-coating solutions to determine their effects upon the resulting coat and upon the time required for coating. Glycerin, propylene glycol, sorbitol, rosin, abitol, zein, as well as several of the polyethylene glycol esters were added in varying concentrations. They were found to be generally unsatisfactory in that they produced either a soft or brittle coat.

Coating Procedure.—The following procedure was found to be the most satisfactory for the application of the Carbowax® coating:

(*a*) The uncoated tablets were placed in the coating pan to the proper depth so that they rolled freely when the pan was in motion.

(*b*) The warm air and exhaust were applied to warm the tablets and remove any dust or loose powder.

(*c*) The application of air was discontinued and a 25% alcoholic solution of Carbowax® 6000 warmed to 50° was applied to the tablets. Enough of this primary coating solution was added to just wet the tablets without an excess. Warm air was applied to help dry the coats.

Successive applications of this solution were made in the same manner until the tablets were evenly coated with a continuous, thin, translucent layer of

[1] Trademark of the Carbide and Carbon Chemicals Corp., New York.

the Carbowax®. This primary stage required approximately one hour for completion.

(*d*) The second stage, consisting of successive applications of a 50% alcoholic solution of Carbowax® at 50°, was carried out in the same manner as the first stage. In all cases, enough solution was applied so as to cover evenly all the tablets without depositing an excess of Carbowax® on the pan. Continued tumbling in the pan appeared to smooth the coat. Warm air was applied to speed the drying of the coats. Sufficient applications were made until the weight of the coat was half that of the tablet. This stage required approximately three to four hours.

(*e*) The final stage, or color coat, was accomplished by the application of successive portions of a colored 40% alcoholic solution of Carbowax® at 50°. The colors were dissolved or dispersed in the solution; either method was found to be successful provided no large particles of color were present to mottle the coating. The application of sufficient color coats to produce smooth, uniformly colored tablets required approximately one hour.

(*f*) The tablets were removed from the pan and placed in an oven at 50–55° for three hours. This warming had a further smoothing effect upon the tablets and when the tablets were allowed to cool to room temperature, the coatings were hard.

(*g*) The final step consisted of the polishing of the tablets. This was accomplished in the usual manner, by two or three applications of a wax solution composed of:

Carnauba wax	200 grains
White wax	100 grains
Carbon tetrachloride, enough to make one pint.	

This solution was applied warm to the tablets while they were tumbling in a canvas-lined pan.

This coating process was successfully employed in the production of a series of differently colored tablets.

Tests.—The Carbowax® coating dissolved completely in five and one-half minutes when tested in accordance with the U. S. P. method.

Samples of the coated tablets were placed in a desiccator at 45° and 100% relative humidity for six weeks. No change was noted in the quality of the coating.

A bottle was half filled with coated tablets and placed on a mechanical shaker for one-half hour. A like amount of sugar-coated tablets was placed in a similar bottle and subjected to the same test. The Carbowax®-coated tablets were unaffected by this treatment while the sugar-coated tablets powdered very perceptibly.

SUMMARY

1. The greatest advantage of this new coating process is the shorter operational time required for the production of satisfactory tablets. This is made possible by the physical nature of the Carbowax®, the use of alcohol as the solvent, and the fewer number of individual applications required.

2. The use of alcohol as the solvent offers another distinct advantage in that the need for protective coatings is eliminated in cases where water would cause an incompatibility.

3. This process provides a noncaloric coating for patients who are forced to restrict their sugar ingestion.

4. The tablets do not lose their luster when handled as do the ordinary sugar-coated tablets.

5. Since it is much simpler, there is much less skill and experience required to employ this procedure successfully, as compared to the sugar-coating process.

6. The coatings are at least as stable, durable, and soluble as sugar coatings.

7. This process can be applied to standard concave as well as deep concave compressed tablets.

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The Use of Polyethylene Glycol as a Binder in Tablet Compression*

By BERNARD MILLER and LEONARD CHAVKIN†

The process developed during this investigation makes it possible to produce pharmaceutically elegant compressed tablets without the necessity for wet granulation. Through the inclusion of powdered polyethylene glycol 4000 in the formula for the tablet, it is possible to compress the properly mixed powdered components into "slugs" which are then screened and recompressed into satisfactory finished tablets. This process has been carried out with the use of a conventional rotary tablet press operating at high speed. This method thus eliminates the need for slow, heavy-duty tablet presses which are currently used in the double compression method for tablet production. It completely eliminates the use of granulating solutions and the lengthy drying process necessary in the wet granulating method and reduces the tendency for "classification" to take place during compression. Thus, the time required for the production of compressed medicinal tablets is considerably shortened and the preparation of a wide range of tablets by the double compression method is made feasible.

THERE ARE two general methods in current use for the production of compressed tablets. The majority of tablets are prepared by the wet granulation method or by double compression, except those made of materials which can be compressed directly.

The object of both methods is to change the powdered tablet components to granules so that uniform, hard, stable tablets can be produced by compression.

The purpose of this investigation was to determine the efficiency and applicability of a new method for the production of compressed tablets wherein a dry binder was used to confer adhesiveness upon the powdered tablet components.

SHORTCOMINGS OF CURRENT METHODS

Wet granulation is the most commonly used technique for the production of tablets since it is applicable to the widest range of materials. However, it involves many distinct operations, some of which require considerable time, resulting in a rather lengthy, complicated process. Since water almost always is a component of the granulating solution, incompatibilities and problems of instability often are encountered which require special procedures for their elimination. Since the drying of the wet granulation is the most lengthy stage of the process, considerable space must be devoted to facilities for drying.

The double compression or "slugging" method offers distinct advantages over the wet granulation process. Since it is a dry method, it simplifies the problem of introducing incompatible or unstable materials, eliminates the wet mixing and subsequent lengthy drying of the components, and reduces the amount of floor space and equipment required for granulation. It involves a considerably shorter time for the successful production of compressed tablets.

There are, however, shortcomings in this method which render it of rather limited applicability and of only secondary importance as a means of tablet making. Tablets made by this method are generally softer than comparable tablets made by the wet granulation process. For the production of useful "slugs" a slow, rugged, heavy-duty tablet press is required. This is expensive in equipment and man-hours necessary for supervision of the machine. In addition, this method is effective with only a limited number of materials which possess the proper physical nature that will allow them to form compact, cohesive aggregates upon the application of pressure. For these reasons, this method is generally regarded as a rather unsatisfactory alternative to the wet granulation method and is usually used for such materials as aspirin which cannot be tableted by wet granulation.

The investigation was based upon the hypothesis that if a dry material could be incorporated into the mixture of powdered tablet components which would increase the adhesiveness of the mixture, satisfactory tablets of a wide variety of medicaments could be produced by either single or double compression on a light-duty rotary tablet machine. This would serve to eliminate the wet granulation method for most materials and broaden the range of usefulness of the "slugging" method.

Such a substance must be relatively nontoxic, compatible with a wide range of medicaments,

* Received December 30, 1953, from the College of Pharmacy, Columbia University, New York, N. Y.
Presented to the Pharmacy Subsection, A. A. A. S., Boston Meeting, December, 1953.
Abstracted from a thesis presented to the Graduate Division of Columbia University College of Pharmacy by Bernard Miller in partial fulfillment of the requirements for the degree of Master of Science.
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and stable under conditions of storage. In addition, it should be white or colorless and not retard the disintegration of the tablet nor the release of the medicament.

The materials selected for this investigation were: glyceryl monostearate,[1] hydrogenated castor oil,[2] Carbowax® 4000,[3] and Carbowax® 6000.[3]

The use of Carbowax® in the formulation of compressed tablets is not unique. Sperandio and DeKay have reported the usefulness of Carbowax® 4000 (60 mesh) as a lubricant in the manufacture of compressed soluble tablets (1). In a subsequent article, Ray and DeKay reported the lubricating efficiency of Carbowax® 6000 (100 mesh) in the formulation of a soluble, compressed tablet composed of varying amounts of lactose, dextrose, and sodium chloride (2). The Carbowax® was found to be most effective in this application when it was incorporated as a powder. Both reports found the optimum concentration of Carbowax® as a lubricant to be 2%.

EXPERIMENTAL

A series of formulations were prepared using a range of concentrations of these dry binders. Various amounts of active ingredients, disintegrator, diluent, and lubricant were used to determine the optimum concentrations of each of these components to produce a properly balanced formula which would yield a satisfactory tablet.

The amount of active ingredient ranged from 0.1 to 0.3 Gm.; binder from 10 to 25%; disintegrator from 10 to 20%; and lubricant from 1 to 3%. The percentages were based upon the weight of the finished tablet. Lactose was used as the diluent wherever one was required to bring the tablet to a reasonable weight. In all instances, dried cornstarch was used as the disintegrating agent and calcium stearate was used as the lubricant.

Theorizing that the particle size of the binder might affect the quality of the tablet, experiments were conducted using binders of various particle sizes. These were obtained by passing the binders through a Fitzpatrick Comminuting Machine at high speed, impact forward, using screens ranging from a No. 3 mesh to No. 80 mesh.

Each of the formulations was prepared with acetophenetidin as the active ingredient by the following procedure:

(*a*) The active ingredient, lactose, starch, and lubricant were weighed and finely powdered. A 20-mesh screen was used to remove any lumps.

(*b*) The binder was passed through the desired mesh of the Fitzpatrick Comminuting Machine.

(*c*) All of the components were mixed in a mass mixer until homogeneity was achieved. The average mixing time was thirty minutes.

(*d*) The powdered mixture was compressed on a Stokes Model B-2 Rotary Tablet Machine at high speed.

[1] Aldo® 33—Glyco Products.
[2] Castorwax®—Baker Chemical Co.
[3] Carbide and Carbon Chemicals Corp.

It was found that the tablets produced by single compression with any of the formulations were soft. Hardness values, using the Monsanto Tablet Hardness Tester, ranged from 1 to 2 Kg. The harder tablets were obtained with high concentrations of dry binder of fine particle size. Capping was frequently observed, although other compression problems were absent.

The tablets resulting from this single compression were passed through a Stokes Oscillating Granulator with a No. 12 mesh screen, and then recompressed. This generally resulted in a harder tablet, the hardness value doubling in almost all cases. The capping, formerly observed, was eliminated.

The binders which gave the best results, based upon hardness values and disintegration rates, were Carbowax® 4000 and Carbowax® 6000 which had been passed through a No. 80 mesh screen. Throughout the experiment there was no noticeable difference between tablets obtained by use of either of these waxes. Carbowax® 4000 was selected arbitrarily for use in the remainder of this investigation.

The formula which was found to be most satisfactory within the limits of this investigation was:

Acetophenetidin	0.3 Gm.
Dried cornstarch	15 %
Carbowax® 4000	20 %
Calcium stearate	2 %

The tablets made with this formula ran smoothly and were hard enough to withstand handling without any evidence of powdering or chipping. The hardness value was 3 Kg. and the disintegration time, U. S. P. method, was twenty minutes.

The effect of heat upon the hardness of the tablets was determined by subjecting them to temperatures ranging from 60° to 110°, in five-degree increments for six hours at each increment. The initial effect of heating was a softening of the tablet and a distinct drop in the hardness value from 3 Kg. at 60° to 1 Kg. at 65° and 0.5 Kg. at temperatures of 70° and above. Upon subsequent cooling to room temperature, however, the tablets became harder than they had been before this treatment. The hardness value increased from 3 Kg. to 5 Kg. after heating for six hours at 65° and cooling to room temperature. No increase over this 5 Kg. value was obtained after heating at any temperature higher than 65°.

Similar formulations with various other active ingredients were prepared. These ingredients were selected since in most cases they had been known to offer some problems when made by the wet granulation method. None of them lends itself to tableting by the usual double-compression method. The amount of diluent, binder, lubricant, and disintegrating agent was varied in each case to suit the requirements of the active ingredient, the final tablet size, and the hardness of tablet desired. In none of these tablets was heat used to increase hardness. Each was found to yield a satisfactory tablet. The specifications for each formula are listed in Table I.

DISCUSSION

An assay of the sodium bicarbonate tablets revealed that they contained 94.4% of the stated amount of active ingredient. This method of compression was unsuccessful for tablets of brewer's

TABLE I.—SOME TABLET FORMULAS PREPARED WITH CARBOWAX® 4000

	Aluminum Hydroxide, Dried Gel	Calcium Lactate	Powdered Thyroid	Ferrous Sulfate, Exsicc.	Sodium Bicarb.	Cascara Sagrada Extract
Weight per tablet, Gm.	0.3	0.3	30 (mg.)	0.3	0.3	0.3
Carbowax® 4000, %	20	20	25	20	15	20
Cornstarch, %	15	15	20	15	10	15
Cal. stearate, %	2	2	2.5	2	2	2
Finished weight, Gm.	0.5	0.5	0.21[a]	0.5	0.44	0.5
Hardness value, Kg.	6	8	4	6	3	3
Disint. time, min.	15	40	75	28	45	20

[a] Lactose 0.09 Gm. added.

yeast, aspirin, and sodium salicylate. In the case of the yeast, the tablets were too soft, even with 20% Carbowax® 4000. Salicylates are known to lower the melting point of Carbowax®, which accounted for the sticking of the mixture to the punches of the tablet machine, as encountered during this investigation.

All of these tablets were compressed on a Stokes B-2 Rotary Tablet Machine at high speed with just enough pressure to form the tablets. Thus the need for a rugged, heavy-duty press was eliminated.

There was no aqueous solution added so that special techniques for introducing incompatible or unstable materials were not necessary. Current procedures for handling incompatible or unstable materials involve the mixing of two separate granulations or the dusting of powdered materials onto blank granulations. In either case the resulting mixture is not homogeneous. Tablets of varying potencies may result as a consequence of the separation of the particles (classification) caused by vibration while in the hopper of the tablet machine. This difficulty was not encountered in this process since the resulting granulation represents a uniform distribution of all the ingredients.

The use of Carbowax® 4000 as a dry binder permits a greater range of medicinals to be tableted by the double compression method. This means a distinct saving in time and equipment necessary for the production of satisfactory compressed tablets.

It was found that the binder should be present within the range of 15 to 25% in order to insure adequate adhesion. Less than 15% binder resulted in tablets which were too soft, and greater amounts produced compression problems. By carrying out disintegration tests on varying amounts of binder, disintegrating agent, and lubricant to produce a properly balanced formula of all the necessary requirements, it was determined that the binder must be within the range of 15 to 25%, the disintegrating agent within the range of 10 to 20%, and the lubricant within the range of 1 to 2.5%, all percentages being by weight and based upon the weight of the finished tablet.

Toxicity and absorption and excretion studies have indicated that the higher molecular weight polyethylene glycols are relatively nontoxic (3–7). It was believed that these reports justified the use of Carbowax® 4000 in this application.

SUMMARY

1. A new method for the production of compressed tablets by the use of polyethylene glycol 4000 as a dry binder has been devised.

2. Several typical formulations have been prepared and tested and optimum percentages of binder, disintegrating agent, and lubricant have been established.

3. This procedure offers the following advantages: (*a*) The lengthy wetting and drying stages of the usual wet granulation process have been eliminated with a resultant marked decrease in the time required to produce compressed tablets. (*b*) The applicability of the double compression method has been extended and the need for a heavy duty slow tablet press has been eliminated. (*c*) Incompatibility and instability due to moisture, as well as the problem of classification, can be eliminated. (*d*) The toxicity of the higher molecular weight polyethylene glycols appears to be low enough to warrant this new application.

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The Chronic Dermal Toxicity of a Series of Polyethylene Glycols*

By THOMAS W. TUSING, JOHN R. ELSEA, and ANITA B. SAUVEUR

Liquid polyglycols E300 and E400 and solid polyglycols E1000, E2000, E4000, E6000, and E9000 were studied over a ninety-day period in rabbits by repeated dermal application. None of the materials produced significant skin reactions or gross evidence of systemic toxicity from prolonged dermal contact. All deaths that occurred during this study of the polyglycols were believed caused by an extraneous parasitic infestation. Liver and kidney function studies on the experimental animals revealed no significant alteration and the values were comparable to those of the controls. There were no histological findings in any of the visceral organs that could be interpreted as being produced by dermal application of the various polyglycol materials. It would appear that these polyethylene glycols would produce no harmful effects when used in applications that might involve prolonged, extensive contact with the human skin.

THE DERMAL TOXICITY and irritating properties of seven polyethylene glycols following prolonged contact with the skin of rabbits have been compared. The materials used were polyglycols with average molecular weights as indicated by the number following the letter "E."

Several investigators have studied the effects of single and repeated applications of various polyethylene glycols to the skin of rabbits. Smyth, Carpenter, and Shaffer (1) found that polyethylene glycols 200, 300, and 400, applied in a single dose of 20 ml./Kg. or repeated dosages (five days a week for eighteen weeks) at 2.0 ml./Kg./day resulted in no systemic injury or skin irritation. Smyth, *et al.* (2), applied polyethylene glycols 1500 and 4000 to the skin of rabbits at dosages of 10 Gm./Kg./week for a total of thirteen weeks and observed no mortality, little dermal irritation, and no noticeable effect on blood urea nitrogen or icteric index values. A review paper by Smyth, *et al.* (3), summarizes the above work, including the oral and parenteral toxicity of polyethylene glycols.

This paper describes the results of repeated skin application to rabbits of seven polyglycols for the purpose of evaluating chronic toxicity. The materials[1] used were polyglycols E300 and E400, which are clear, colorless, viscous liquids, soluble in water, and polyglycols E1000, E2000, E4000, E6000, and E9000, which are white water-soluble, wax-like solids.

EXPERIMENTAL

Procedure.—Male and female albino rabbits, weighing between 1.0 and 2.0 Kg. were divided at random into eight groups of ten rabbits each. Of the eight groups of rabbits, one group served as controls and the remaining seven, designated as experimental groups, received the various polyglycols over a ninety-day period.

Initially, and one or more times each week, the abdominal fur was closely clipped from each animal to expose the abdominal skin area for contact with the experimental material. The rabbits were individually housed with food and water available at all times.

The ten control animals were subdivided into two groups of five rabbits each; one control group served as controls for the animals receiving daily applications of the liquid polyglycols, E300 and E400, and the other group served as controls for the animals receiving weekly applications of the solid polyglycols, E1000, E2000, E4000, E6000, and E9000. The control animals were handled and bandaged in the same manner as the experimental animals, except that binders only were applied to the skin. For the purposes of this paper, the daily or weekly handling of the control animals was counted as daily or weekly application.

Ten animals each received 2.0 ml./Kg. of liquid polyglycol E300; ten animals each received the same dosage of liquid polyglycol E400. The material was gently applied by inunction to approximately 250-cm.^{2} of the abdominal skin of each animal. The treated area was covered with a gauze binder which was secured to the trunk of the animal with adhesive tape. After approximately twenty-two hours, the binders were removed and the animals observed for signs of systemic toxicity and dermal irritation. Daily applications were made in this manner five days a week for a period of thirteen weeks.

Each of the solid polyglycols, E1000, E2000, E4000, E6000, and E9000, were applied to the abdominal skin areas of each of ten rabbits at a dosage level of 10 Gm./Kg. once weekly during a thirteen-week period. Approximately 250 cm.^{2} of skin was covered by the dose. This dosage of the respective material was applied on the first day of the week and remained on each animal for five consecutive days. The different physical consistencies of the solid polyglycols made it necessary to alter the technique for the weekly application. Polyglycols E1000 and E2000 were placed on a water bath and melted at between 40° and 50° and then spread on nonabsorbent paper backing and allowed to cool. Polyglycols

* Received December 10, 1953, from the Hazleton Laboratories, Falls Church, Va.

[1] The materials were supplied by The Dow Chemical Company, Midland, Mich., sponsor of this investigation.

E4000, E6000, and E9000 were each mixed with 6.0 ml. of water per 10 Gm. of material, the mixture placed on a water bath at 40–50° until a smooth, homogeneous paste formed, and the paste spread on a nonabsorbent paper backing and allowed to cool. The paper backing holding the respective content of polyglycol was held in place with gauze binders secured with adhesive tape. After the material had been held against the abdominal skin for five days, the gauze binders were removed and any material remaining on the skin was sponged off with warm water. The animals were then weighed and examined for two consecutive days for signs of systemic toxicity and dermal irritation before re-applying the material.

After twelve weeks, a representative number of control and experimental animals were sacrificed and blood and urine specimens collected for the determination of blood urea nitrogen, icteric index, and urinary urobilinogen. After thirteen weeks the remaining control and experimental animals were sacrificed and autopsied. Representative tissues from the animals were selected and preserved in formalin. Histological examination was made of the thyroid, adrenal, liver, kidney, urinary bladder, skin, and testis or ovary from representative animals.

Results and Discussion.—None of the polyglycols used in this study produced significant skin reactions. Occasionally a mild erythematous reaction was observed, but this was interpreted as being associated with the daily or weekly handling of the animals, since similar reactions were observed among the control animals. Histological examination of skin sections from the test animals revealed slight changes consistent with mild irritation; similar changes were also found in an occasional control animal.

There was no evidence of systemic toxicity observed among any of the test animals which could be associated with the application of the various polyglycols; there were, however, fourteen deaths among the test animals and none among the control animals during the experimental period. These deaths were probably caused by incidental coccidial infection demonstrated in some animals at necropsy. On termination of the experiment the finding of heavy parasitic infestation among surviving control and test animals substantiates the probable cause of death in the animals that died. In spite of the stress imposed by systemic parasitism, the animals exhibited normal behavior and had good body weight gains.

Autopsies performed at termination of the experiment revealed no specific gross pathology which could be attributed to the dermal application of the various polyglycols. In both control and test groups coccidia and larval tape worm infestations were prevalent and involved the liver, intestine, and to some extent, the kidneys.

Histological study of the livers and kidneys of all surviving animals and of thyroid, adrenal, spleen, urinary bladder, and testis or ovary from three rabbits of each control and test group revealed no specific cellular change that could be attributed to the dermal applications of the various polyglycols.

At the time of autopsy, blood and urine specimens were collected for analysis from at least five animals of each test and control group. The blood urea nitrogen values and icteric index values of the test animals were within normal limits and comparable to those of the control animals. All of the urine samples tested were negative for urinary urobilinogen at a 1:20 dilution. These clinical laboratory findings indicate that none of the polyglycols tested interfered with kidney function or altered liver function.

The above findings differ from those observed by Luduena, *et al.* (4), following repeated dermal application by inunction of polyethylene glycols 200 and 400 and Carbowax® 1500 and 4000 to rabbits six days a week for five weeks. Their work indicated that dietary limitations may influence the toxicity of polyethylene glycols applied dermally, but as no control animals were used in the study, their findings are difficult to evaluate.

The results with polyglycols are in general agreement with those of other investigators (1), (2), and (3).

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WHO MAKES IT?

The Registry of Rare Chemicals, Armour Research Foundation of the Illinois Institute of Technology, 35 West 33rd Street, Chicago 16, Ill., seeks information on sources of supply of the following chemicals:

Zinc hydride
Titanium trifluoride
Sodium telluride
Phosponium iodide
Molybdenum carbonyl
3-Methyl-1,6-hexandiol
Dimethylmaleic anhydride
Ethyl *alpha*-methylacetoacetate
omega-Chloro-2,3,4-trihydroxyacetophenone
6,7-Dihydroxycoumaran
2,4-Dichlorophenylsulfoneacetic acid
Gentisic aldehyde
3-Methyl-2-naphthoic acid
1,2,4,5-Tetrahydroxybenzene
6-Hydroxynicotinamide
Melibionic acid
Creatininease
Tigonin
Sedo-heptulose
Carbonic anhydrase

Formation of Hydrogen Peroxide in Calamine Lotions*

By DAVIS R. REESE† and EARL P. GUTH‡

A study of eight official and nonofficial calamine lotions is presented. Hydrogen peroxide formation is accomplished by irradiation with ultraviolet light from several sources, both natural and artificial. A comparison of the bacteriostasis from the irradiated and nonirradiated lotions is given, using an agar cup technique.

ALTHOUGH chemists (1–9) have studied the formation of hydrogen peroxide from irradiated zinc oxide for over twenty-five years, apparently no one has attempted to investigate the pharmaceutical aspects of this phenomenon. The present investigation includes a comparative study of the effect of irradiating various calamine lotions with an ultraviolet lamp, sunlight, incandescent lamp, and fluorescent lamp. In addition, the bacteriostatic actions of these lotions have been studied, using an agar cup technique.

There are two natural characteristics of calamine lotions which make them advantageous for a basic study. First, they are aqueous suspensions—water being necessary for the production of the hydrogen peroxide, while zinc oxide serves as a photosensitizer. Second, they often contain phenol and other easily oxidizable organic materials which, as found by previous workers (5), increases the value of zinc oxide as a photosensitizer.

In photochemical sensitization, a system not sensitive to light becomes sensitive upon the addition of a small quantity of another substance (sensitizer). The sensitization occurs only for the wavelengths of light absorbed by the added sensitizer (10). For zinc oxide, this limits the effective light to those wavelengths less than approximately 400 mμ (2). Only small percentages of sunlight, incandescent light, and fluorescent light fall into the required range (11, 12), but these are sufficient to stimulate the production of measurable quantities of hydrogen peroxide under proper conditions.

A survey of the literature discloses in addition to the above facts the following points which are of importance when considering pharmaceutical preparations of zinc oxide: (*a*) of the known effective organic materials (additives) which increase the peroxide yield, only phenol and glycerin have been used to any appreciable extent with zinc oxide, (*b*) the process of manufacture of zinc oxide determines its ability to catalyze the photochemical formation of hydrogen peroxide, and (*c*) oxygen plays an important role in the reaction.

* Received December 23, 1953, from The Ohio State University College of Pharmacy, Columbus, Ohio.

Abstracted in part from a thesis presented to the Graduate School of The Ohio State University by Davis R. Reese in partial fulfillment of the requirements for the degree of Master of Science.

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EXPERIMENTAL

Apparatus.—*Ultraviolet sources.*—(*a*) Dazor "Floating Fixture" ultraviolet lamp Model U-58 equipped with a General Electric UA-3 Uviarc tube, (*b*) Dazor "Floating" Universal Model fluorescent lamp equipped with two 15-watt 45° White CBF DxC Duro-Test tubes, (*c*) Champion 200-watt incandescent bulb, and (*d*) sunlight.

Irradiation Site.—An aluminum water bath was constructed and fitted with a plywood cover (covered with aluminum foil) containing two holes, each the size of a 250-ml. beaker. By using the cover, the beakers, containing the samples, were centered over magnetic stirrers and at the same time centered 15 cm. under the light source which had been clamped into position. A grooved rack was built which enabled the operator to slide the water bath from under the lamp.

Materials.—(*a*) American process zinc oxide U. S. P.[1] This zinc oxide was used in all lotions. (*b*) French process zinc oxide U. S. P.;[2] an acicular zinc oxide was used only in a preliminary study.

PROCEDURE

Irradiation.—*Ultraviolet Lamp.*—Five grams of the lotion was diluted with 20 ml. of distilled water. Where solids such as zinc oxide and calamine were irradiated, 25 ml. of water was used. The beakers containing the prepared samples were then covered with Pyrex glass Petri dishes (supported to permit air circulation). The samples were irradiated for equal periods of time in each of the two positions in the water bath to neutralize any possible difference in ultraviolet light. The samples were then centrifuged and the centrifugates assayed iodometrically for hydrogen peroxide.

Incandescent Light.—The ultraviolet lamp was replaced by two 200 watt bulbs, i. e., one over each beaker. Except for the irradiation period, which was four hours, all conditions remained as given above.

Fluorescent Lamp.—The samples were placed 8 cm. from the light source in uncovered Petri dishes. Corrugated board was used to exclude external light. At the end of four hours the samples were removed, centrifuged, and assayed.

Sunlight.—Five-gram samples plus 10 ml. of water were placed in direct sunlight in uncovered Petri dishes. Additional water was added as re-

[1] Merck and Company, Rahway, N. J.

[2] Duff Chemical Company, New York, N. Y.

TABLE I.—IRRADIATION OF VARIOUS INGREDIENTS OF CALAMINE LOTIONS[a] (HYDROGEN PEROXIDE IN MICROGRAMS)

Ingredient	Irradiation Period, Min.	Irradiated, μg.	Non-irradiated, μg.	Peroxide Found, μg.
Phenolated calamine lotion N. F. VIII (without zinc oxide or calamine) (5 Gm.)	60	9	5	+ 4
Zinc oxide, Merck (0.514 Gm.)[b]	60	90	10	+ 80
Zinc oxide, Merck (0.20 Gm.) + phenol (1%)	30	1,066	10	+1,056
Zinc oxide, Merck (0.20 Gm.) + phenol (1%)	60	1,407	10	+1,397
Zinc oxide, ZoCo (0.20 Gm.) + phenol (1%)	30	627	5	+ 622
Zinc oxide (99%) + red ferric oxide (1%) (0.520 Gm.)[b]	60	38	22	+ 16
Zinc oxide (97%) + red ferric oxide (3%) (0.530 Gm.)[b]	60	16	16	0
Zinc oxide (99%) + yellow ferric oxide (1%) (0.520 Gm.)[b]	60	10	19	− 9
Zinc oxide (96%) + yellow ferric oxide (4%) (0.536 Gm.)[b]	60	7	18	− 11
Zinc oxide (93%) + red ferric oxide (3%) + yellow ferric oxide (4%) (0.553 Gm.)[b]	60	6	17	− 11
Zinc oxide (93%) + red ferric oxide (3%) + yellow ferric oxide (4%) (0.553 Gm.)[b] + phenol (1%)	60	1,440	17	+1,423

[a] In making interpretations from this table, careful attention must be given to the quantity of ingredient used, as well as the period of irradiation.
[b] Each of these contains 0.514 Gm. of zinc oxide.

quired (identical amounts to each sample) and after four hours (10:30 a. m. to 2:30 p. m.) the samples were removed from the sunlight, centrifuged, and assayed.

Hydrogen Peroxide Detection and Determination.—Titanium sulfate (13) was used as a specific qualitative test for hydrogen peroxide, while the iodometric method of Chari and Qureshi (5) was used as the quantitative test, employing a 10-ml. microburet.

Bacteriostatic Study.—Agar cup-plates were prepared containing 25 ml. of 3.7% brain heart infusion and 1.5% agar. The cups were 18 mm. in diameter and contained 0.5 cc. of lotion as measured from a 1-cc. hypodermic syringe. Pure cultures of recently isolated coagulase positive staphylococci were used as the test organisms.

RESULTS

Irradiation of Various Ingredients of Calamine Lotions.—None of the ingredients (with the exception of zinc oxide) of the various lotions used in this investigation showed more than a trace (less than 5 μg.) of hydrogen peroxide when irradiated individually with water for one hour. As shown in Table I, definite peroxide formation occurred when plain zinc oxide was irradiated. A greatly increased amount of hydrogen peroxide was formed when 1% phenol was added. The French process zinc oxide proved to be only 60% as effective as the American process zinc oxide in peroxide formation, although they were both U. S. P. grade. Ferric oxide had a marked inhibiting action unless phenol was present. Addition of 1% yellow ferric oxide or more than 3%

TABLE II.—HYDROGEN PEROXIDE ACTIVITY OF CALAMINE LOTIONS (IN MICROGRAMS)

	Lotion		pH	Non-irradiated, μg.	UV Lamp, 1 hr.,[e] μg.	Sunlight, 4 hr.,[f] μg.	Cloudy Sky, 4 hr., μg.
A	Calamine lotion	U. S. P. XIII	10.5	36	+ 91	+ 156	...
B	Phenolated calamine lotion	N. F. VIII	8.2	26	+1,123	+1,477	+1,712
C	Calamine lotion	U. S. P. XIV	6.5	13	+ 594	+ 692	...
D	Phenolated calamine lotion	N. F. IX	6.7	13	+ 988	+ 455	...
E	Neocalamine lotion	N. F. IX[a]	..	27	− 18	...	...
F	Neocalamine lotion	N. F. IX[b]	7.7	21	− 9	− 15	...
G	Phenolated calamine lotion	N. F. IX	7.6	22	+1,212	+ 870	...
H	Calamine lotion	O. S. U.[c] (1 year)[d]	6.9	11	+ 875	...	...
I	Phenolated calamine lotion	O. S. U. (1 year)	6.8	25	+1,200	...	...
J	Calamine lotion	O. S. U. (1 month)	..	0	+ 212	...	...
K	Phenolated calamine lotion	O. S. U. (1 month)	..	3	+ 548	...	+ 823
L	Calamine lotion	O. S. U. (fresh)	7.0	0	+ 154	...	...
M	Phenolated calamine lotion	O. S. U. (fresh)	7.0	0	+ 439	...	...
N	Calamine lotion	O. S. U. (2 weeks)	7.0	7	+ 57	+ 24	...
O	Phenolated calamine lotion	O. S. U. (2 weeks)	7.0	16	+ 156	+ 160	+ 123

[a] Using Mallinckrodt prepared neocalamine.
[b] Using Merck zinc oxide + red and yellow ferric oxide.
[c] Prepared from the water-dispersible calamine powder as reported by Celmins, Cosgrove, and Guth.[14]
[d] Lotions *H*–*M* were made with the same powder base (prepared with a micropulverizer). Lotions *N* and *O* were made with the same powder base (prepared with a hand sieve).
[e] Temperature varied from 35° to 40°; light intensity varied from 550 to 600 foot candles (using a Kodak 18% neutral test card).
[f] Temperature varied from 24° to 25°; light intensity varied from 75 to 125 foot candles (using a Kodak 18% neutral test card).

red ferric oxide resulted in a net loss of hydrogen peroxide production, thus neocalamine was a poorer peroxide producer than calamine when they were used as plain lotions (Table II).

Effect of Irradiation Time on the Formation of Hydrogen Peroxide.—To determine the best irradiation time, samples containing 0.2 Gm. of zinc oxide and 1% phenol were irradiated with ultraviolet light. The following average amounts of hydrogen peroxide were formed:

120 minutes	2.19 mg.
90 minutes	1.91 mg.
60 minutes	1.49 mg.
45 minutes	1.20 mg.
30 minutes	0.77 mg.
15 minutes	0.47 mg.

This experiment showed that the formation of the peroxide is not linear, one hundred twenty minutes of irradiation yielding less than five times the amount of peroxide formed in fifteen minutes.

Irradiation of Lotions with the Ultraviolet Lamp.—The dilution of the lotions with water was necessary to make possible the use of magnetic stirrers. The action of the stirrers was twofold, they permitted more of the lotion to be exposed to the light and they also permitted continuous contact with air (oxygen).

A summary of the results of sixty minutes of irradiation is found in Table II. An analysis of these figures reveals several facts:

(*a*) Neocalamine lotion N. F. IX either forms no peroxide or, if any peroxide is formed during irradiation, it is destroyed before it can be measured. The mechanism for the formation of hydrogen peroxide by zinc oxide apparently is not permanently destroyed, for the addition of 1% phenol transforms this lotion into a hydrogen peroxide producer.

(*b*) The plain lotions vary widely in their hydrogen peroxide activity. It appears therefore, that some of the ingredients are acting as "additives" and that this capacity is variable. The O. S. U. lotion (14), when freshly made, has a relatively small peroxide activity, whereas by allowing this lotion to age for one month prior to irradiation a 25% increase in peroxide is noted; there is an even greater increase if the lotion ages for one year prior to irradiation.

(*c*) In each case, the addition of phenol to a lotion results in increased peroxide formation. Lotions *B*, *G*, and *I* are practically the same, while lotion *D* is only slightly less, indicating that the addition of phenol results in a maximum peroxide activity which varies only slightly from lotion to lotion.

Irradiation of Lotions with Sunlight.—The results, as shown in column 4 of Table II, are very erratic. Lotions *A*, *B*, *C*, and *O* show higher peroxide activity in sunlight (4 hours) than under the ultraviolet lamp (1 hour), which is plausible, since the peroxide formation depends on the amount of usable ultraviolet light. Lotion *F* (neocalamine), as with the UV lamp, shows a loss of hydrogen peroxide when irradiated, while lotions *D*, *G*, and *N* form less peroxide in sunlight than under the ultraviolet lamp. No satisfactory explanation has been found for action of lotions *D*, *G*, and *N*.

Lotion *B*, in addition to being air cooled, was also water cooled during sunlight irradiation. Although the temperature of the water bath was 32° in contrast to the 37° average (35–40°) with the air-cooled samples, no appreciable difference was found in the amount of hydrogen peroxide formed.

Irradiation of Lotions under Cloudy Sky.—Contrary to expectations, as shown in column 5 of Table II, of the three lotions irradiated under cloudy sky, two formed more peroxide than when irradiated with the UV lamp, while one formed more than when it was irradiated in direct sunlight. Luckiesh (15) states that ultraviolet light is decreased by clouds even more than is visible light, therefore, some factor other than ultraviolet light could be responsible for the difference in peroxide activity found under cloudy and clear atmospheric conditions. The temperature remained almost constant (24.5°) during the cloudy irradiation. As was shown with sunlight, a drop from 37° to 32° had no appreciable effect on peroxide formation, yet apparently a drop from 32° to 25° does have an effect. This difference in temperature could have affected the actual formation of the hydrogen peroxide, or the stability of the peroxide, or the solubility of the peroxide.

Irradiation with a Fluorescent Lamp.—Phenolated calamine lotion N. F. VIII was irradiated for four hours with a twin-tubed fluorescent desk lamp. This resulted in the formation of 315 μg. of hydrogen peroxide or 21% of the amount formed in direct sunlight, indicating that artificial light can be effective in the formation of hydrogen peroxide.

Irradiation with an Incandescent Lamp.—Phenolated calamine lotion N. F. VIII was irradiated with a 200-watt incandescent bulb for a period of four hours, with the formation of 374 μg. of hydrogen peroxide or 25% of that formed by direct sunlight.

It is significant that lotions *H* and *I*, which had been stored on the laboratory shelf for a period of one year, upon analysis showed only small contents of hydrogen peroxide. Lotion *H* was stored in a clear glass bottle, while lotion *I* was in an amber container.

Bacteriostatic Study.—The only evidence of the bacteriostatic action of calamine lotions was presented by E. Meyer (16), and this was done indirectly by plating the organisms remaining on the skin after the use of calamine lotion. Kavanagh (17) has shown that hydrogen peroxide can be bacteriostatic in concentrations of from 4 to 31 μg./ml., which in most instances would be much smaller quantities than have been formed by irradiation of the lotions.

A portion of each lotion was irradiated with the ultraviolet lamp, the remainder remaining unirradiated. No irradiation periods are given, since in one instance seventy-two hours elapsed between irradiation and use; the peroxide content of the lotion, however, was determined at the time of its use. In the case of lotion *B*, a known hydrogen peroxide solution was prepared containing approximately the same concentration as did the irradiated lotion. This known peroxide solution was tested both *per se* and with 1% phenol.

The lotions were tested under three sets of conditions (see Table III). Actually, lotions *A* and *B* were run as one study, while lotions *C*, *D*, *H*, and *I* were run as a second study.

Lotions *A* and *B* are calamine lotion U. S. P. XIII and phenolated calamine lotion N. F. VIII. It is apparent that the U. S. P. XIII lotion exhibits no

bacteriostatic action when used in the dark, but upon irradiation a definite zone of inhibition is produced. The addition of phenol to the nonirradiated lotion results in good inhibition, but even this zone can be increased by irradiation of the phenolated lotion. The bacteriostatic action of phenol and hydrogen peroxide combinations apparently show synergism, for the sum of the combination (6.8 mm.) is less than the combined effects of the single agents, i. e., phenol (4.6 mm.) hydrogen peroxide (3.9 mm.).

Calamine lotion U. S. P. XIV and the plain O. S. U. lotion show a very slight bacteriostatic action when used without irradiation. The other results, with the lotions incubated at 37°, are the same as for lotions *A* and *B*, that is, the phenolated lotions exert definite bacteriostatic action and all zones of inhibition are greater for the irradiated lotions when compared with the corresponding nonirradiated ones.

2. Plain calamine lotions vary widely in their hydrogen peroxide activity, depending upon the ingredients present.

3. Ferric oxide inhibits hydrogen peroxide formation unless "additives" are present.

4. The formation of hydrogen peroxide is apparently not linear with respect to time.

5. There is some indication, that aging may increase the ability of a zinc oxide lotion to form hydrogen peroxide upon irradiation.

6. Sunlight is effective in promoting hydrogen peroxide formation. Apparently this is but little changed during cloudy weather.

7. Fluorescent and incandescent lamps are both usable sources of ultraviolet light, and if used over prolonged periods of time and in close proximity to zinc oxide lotions, will produce hydrogen peroxide.

8. Calamine lotions, in themselves, are not effective bacteriostatic agents. The addition of one per cent phenol and/or irradiation with ultraviolet light provides lotions which are bacteriostatic. The phenol and hydrogen peroxide (formed by irradiation) appear to show complemental synergism.

9. Under normal storage conditions, glass containers would appear to prevent hydrogen peroxide formation in calamine lotions.

10. Assuming that the formation of hydrogen peroxide makes a therapeutic contribution to calamine lotions, it would seem that clothing should not be placed over the area covered by the lotion, since hydrogen peroxide appears to be formed only by exposure of zinc oxide to light.

TABLE III.—BACTERIOSTATIC ACTION OF IRRADIATED AND NONIRRADIATED CALAMINE LOTIONS (INHIBITION ZONES IN MM. USING 1/2 CC. OF LOTION)[a]

		Peroxide Content (per 1/2 Gm.), μg.	37° in the Dark, mm.		Sunlight,[b] mm.		Diffused Light,[c] mm.	
			Non-irrad.	Irrad.	Non-irrad.	Irrad.	Non-irrad.	Irrad.
A	Calamine lotion U. S. P. XIII	9	0.0	1.9				
B	Phenolated Calamine lotion N. F. VIII	104	4.6	7.1				
	Hydrogen peroxide[d] (lotion *B* only)	96	3.9					
	Hydrogen peroxide + phenol (1%) (lotion *B* only)	95	6.8					
C	Calamine lotion U. S. P. XIV	48	0.0	1.3	1.1	2.5	0.0	2.1
D	Phenolated calamine lotion N. F. IX	24	1.0	1.5	2.0	2.4	1.8	2.0
H	Calamine lotion O. S. U.	16	0.0	1.2				
I	Phenolated calamine lotion O. S. U.	33	2.9	3.2				

[a] The larger zones for lotions *A* and *B* are not indicative of better bacteriostatic action, since the culture used was not a fresh transfer as with the other lotions.

[b] Reflected sunlight (from white concrete) for 3 hours (3:30 to 6:30 p. m.), then incubated at room temperature for 21 hours. The term "nonirrad." in this column means that the lotion was not irradiated with the ultraviolet lamp before being placed in the agar cup.

[c] Diffused light of the laboratory was allowed to enter the bottom of the plates for 24 hours. "Nonirrad." has the same connotation as in the Sunlight column.

[d] A known hydrogen peroxide solution containing approximately the same amount of peroxide as the irradiated lotion *B*.

Only lotions *C* and *D* were tested in sunlight and diffused light. It is very important to note that sunlight, even though reflected from a concrete surface and passing through Pyrex glass, is still capable of forming enough hydrogen peroxide in the plain lotion to give it bacteriostatic activity. Conversely, under similar conditions, diffused room light seems incapable of forming peroxide in bacteriostatic quantity. This would seem to mean that to obtain hydrogen peroxide activity from zinc oxide by artificial light (fluorescent and incandescent), it is probably necessary to have direct exposure to the rays, whereas, for sunlight even the indirect rays seem satisfactory.

SUMMARY AND CONCLUSIONS[1]

1. U. S. P. zinc oxide may vary in its peroxide activity, depending on its source.

[1] Further investigation of this problem is in progress. Additional reports will be presented as the data warrant.

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Excretion of Iodinated Compounds Administered to Mice*

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A study of the excretion of iodinated compounds is described which involves the chemical determination of iodine in the urine, bile, and gastrointestinal tract of the mouse. Values obtained following doses of cholecystographic and pyelographic agents used clinically are presented. Results from other iodinated compounds indicate that prediction of predominant excretory pathways based on chemical structures is difficult. However, following most of the mono benzene types usually only traces of iodine appear in the bile, whereas about 50% of the administered dose is found in the urine. On the other hand, compounds containing two benzene rings favor less urinary iodine but relatively greater amounts in the gall bladder provided certain additional chemical groups are also present. There appears to be no correlation between rapidity of passage along the gastrointestinal tract and channel of excretion.

IODINE has long been used as a tracer in the study of excretory routes and radiopaque qualities of various chemicals containing different molecular structures (1–4). Most interest has centered around the properties of compounds having the iodine bound to a carbon atom (5, 6). In aromatic compounds such a bond is quite stable under most metabolic conditions in the body, and by the ease with which the iodine can be identified, allows estimates to be made of the distribution of the molecule in the body.

In the present study the absorption, distribution, and excretion of a series of iodinated compounds have been examined in the mouse since it has been found that this animal may be conveniently used in such studies (7).

EXPERIMENTAL

The drugs were introduced either orally by stomach tube or intravenously into the tail veins of the mice. An 0.8% solution of Na_2CO_3 was used as a convenient vehicle in which to administer the compounds being tested. These were given in such a concentration that the dosage of 0.5 ml. per 20-Gm. weight contained usually either 50 mg. or 100 mg. of iodine per Kg. body weight.

Male mice (Carworth Farms CF-1 Strain) weighing between 15 and 30 Gm. were divided into groups of 10, dosed, and placed in cylindrical wire cages without food or water. The cages were supported by fine wire-mesh screens which covered large glass funnels leading to small beakers which collected the urine voided during the experiment. Environmental temperature was kept between 22° and 25°. After three hours the animals were deeply anesthetized with Evipal® sodium to avoid loss of urine during convulsive spasms. The dose of the anesthetic was 0.5 ml. per 20 Gm. mouse of a 1% solution intraperitoneally. The abdomens were opened and the urine was aspirated from each bladder with a small syringe, pooled with that in the beaker, and the volume was measured. This volume varied between 3 ml. and 9 ml. for each group. The wire screen and funnel were washed with distilled water and the washings were added to the urine already collected. The pooled samples were diluted to 250 ml. or to a volume which contained approximately 10 μg. of iodine per ml.

The neck of the gall bladder was grasped with a small pair of forceps and the cystic duct cut. The

* Received Dec. 1, 1953, from The Wellcome Research Laboratories, Tuckahoe, N. Y.

ligament connecting the gall bladder to the diaphragm was severed and the organ then removed. The gall bladders from a single group were transferred to a test tube and dissolved by adding 0.5 ml. of 2 *N* NaOH. After boiling for ten minutes in a water bath the solution was cooled, partly neutralized with 0.8 ml. of *N* HCl and diluted to 25 or 50 ml. so that 1 ml. was expected to contain about 10 μg. of iodine.

The iodine in both the urine and bile solutions was determined by the method of White and Rolf (8). In carrying out this determination, it is essential to remove all MnO_2 formed during the oxidation by shaking vigorously with a sufficient excess of $NaNO_2$. The excess nitrite must be completely destroyed with urea before the final titration. The results for each group were usually expressed as mg. of iodine per Kg. of body weight. The iodine contents of various portions of the gastrointestinal tract were also determined after homogenizing the tissues in a Waring Blendor and making suitable dilutions of the resultant material. These results were expressed in percentages of the administered doses.

The acute LD_{50} values were determined in male mice by administration of single oral doses, the insoluble compounds being given in 2% starch suspension. These values are included for information as to toxicity in relation to chemical structure and are reported as Gm. of compound per Kg. body weight.

TABLE I.—REPRODUCIBILITY OF IODINE RECOVERY IN BILE FOLLOWING ORAL IODOALPHIONIC ACID ADMINISTRATION

Test	50 mg. Iodine per Kg. Dosage	100 mg. Iodine per Kg. Dosage
	I_2 Found, mg./Kg. Body Wt.	
1	1.26	1.88
2	1.47	2.38
3	1.57	1.92
4	1.65	2.67
5	1.09	1.44
6	0.95	1.99
	I_2 Found, mg./Gm. gall bladder	
1	2.10	2.91
2	1.69	2.95
3	1.75	3.01
4	1.67	3.10
5	1.67	3.17
6	1.83	3.58

RESULTS AND DISCUSSION

Preliminary studies using iodoalphionic acid indicated that the method used for determining the iodine content was quite reproducible. This was tested by administering oral doses of 50 mg. and 100 mg. per Kg. body weight of iodine equivalent to several groups of 10 mice each. The results of six assays on gall-bladder contents are given in Table I. An even greater precision was obtained by having the results in terms of gall-bladder weight, but this involved the additional weighing of the gall bladders. (See Table I.) These determinations were made three hours after dosing because it was found in other tests that maximum excretion occurred at that time. This is illustrated in Fig. 1. However, it cannot be assumed that all compounds will reach a maximum excretory level at the three-hour period.

To obtain information as to the shape of the dose-response curve a series of four graded doses of the above compound representing 25, 50, 100, and 200 mg. of iodine per Kg. respectively were given to different groups of mice and the iodine contents of the gall bladders determined at the end of three hours. When the logarithm of the iodine content was plotted against the logarithm of the dose a straight line was obtained as shown in Fig. 2. Similar studies on urinary excretion values were conducted with iodopyracet being used as a reference material. See Fig. 3.

As shown in Table II, marked differences were obtained in the iodine excretion of known cholecystographic and pyelographic agents. The first two compounds listed, tetraiodophenolphthalein and β-(4-hydroxy-3,5-diiodophenyl)-α-phenyl-propionic acid (Priodax®), were found in the bile at levels of 1 to 3 mg. of iodine per Kg. of body weight. By contrast with these two clinical cholecystographic agents, pyelographic drugs as exemplified by 3,5-diiodo-4-pyridone-N-acetic acid diethanolamine (Diodrast®) and sodium *ortho*-iodohippurate (Hippuran®) were virtually absent from the bile. Two other pyelographic compounds, disodium-N-methyl-3,5-diiodo-chelidamate (Neo-Iopax®) and methiodal sodium (Skiodan®) likewise did not appear in the bile. On the other hand, the pyelographic agents Diodrast® and Hippuran® appeared in the urine in very much greater amounts than the two cholecystographic substances. Although the actual levels of excretion of the latter two substances in the urine were low, Priodax® was definitely higher than tetraiodophenol-

TABLE II.—COMPARISON OF COMMON RADIOPAQUES

Compound	Dosage, mg. I_2/Kg.	Excretion, mg. I_2/Kg. After Oral Dosing: Gall Bladder	Urine	After I. V. Dosing	LD_{50} Oral, Gm./Kg.
Tetraiodophenolphthalein	50	1.06	0.56	..	0.36
	100	1.48	0.73	..	
β-(4-hydroxy-3,5-diiodophenyl)-α-phenyl-propionic acid (Priodax®)	50	1.46	4.8	..	4.0
	100	2.48	12.9	..	
Sodium ortho-iodohippurate (Hippuran®)	50	Trace	23.0	33.7	>10.0
	100	Trace	41.8	66.1	
3,5-diiodo-4-pyridone-N-acetic acid diethanolamine (Diodrast®)	50	Trace	17.1	32.4	>15.0
	100	Trace	38.5	64.7	
Disodium-N-methyl-3,5-diiodochelidamate (Neo-Iopax®)	50	0	2.54	28.6	>15.0
	100	0	3.56	76.8	
Methiodal sodium (Skiodan®)	50	0	23.6	22.9	>15.0
	100	0	44.9	67.7	

TABLE III.—EXCRETION OF IODINE IN GALL BLADDER AND URINE FOLLOWING ORAL ADMINISTRATION OF COMPOUNDS HAVING ONE OR NO BENZENE RING

Compound Number	Compound	Recovered I_2 (mg./Kg. Body Weight) Gall Bladder 50 mg. I_2 per Kg. Dose	Gall Bladder 100 mg. I_2 per Kg. Dose	Urine 50 mg. I_2 per Kg. Dose	Urine 100 mg. I_2 per Kg. Dose	LD_{50} Oral, Gm./Kg.
50–10	Sodium iodide	0.06	0.29	22.8	37.6	1.1
49–193	*o*-Iodoanisole	0.20	0.37	28.2	51.7	>2.0
49–190	2,4,6-Triiodophenol	0.36	0.59	5.6	16.0	>4.0
1001	Triiodoresorcinol	0.19	0	7.0	20.2	4.5
49–368	2,4,6-Triiodo-phenoxy acetic acid	0.57	0.89	2.9	3.5	0.75
1008	Sodium *o*-iodobenzoate	0.04	0.06	30.8	55.7	1.5
1009	Sodium *m*-iodobenzoate	0.12	0.17	25.3	61.8	2.0
1010	Sodium *p*-iodobenzoate	0.21	0.45	27.7	37.3	2.5
49–65	Sodium 2,5-diiodobenzoate	0.55	1.09	19.0	46.6	1.5
49–195	2,3,5-Triiodobenzoic acid	0.85	1.71	5.7	7.5	1.2
49–196	3,5-Diiodo-2-hydroxybenzoic acid	0.38	0.65	1.7	2.5	1.0
49–194	3,5-Diiodo-4-hydroxybenzoic acid	0.29	0.39	31.3	53.3	4.0
49–279	3,5-Diiodo-4-aminobenzoic acid	..	0.85	...	40.5	>4.0
1007	2,5-Diiodohippuric acid	Trace	Trace	23.5	56.1	>4.0
49–187	3,5-Diiodo-*l*-tyrosine	0	0	10.3	20.5	>4.0
46–88	N-methyl-N(4-hydroxy-3,5-diiodophenethyl)-β-alanine	0.38	..	18.2	..	(0.58 i. p.)
49–186	Sodium tetraiodophthalate	Trace	Trace	1.0	2.8	3.0
1005	Sodium *m*-iodobenzenesulfonate	0.27	0.06	17.5	35.1	5.0
1004	Sodium *p*-iodobenzenesulfonate	0.03	0.04	24.6	44.0	5.0
1003	Potassium 3,4-diiodobenzenesulfonate	0.62	0.16	13.7	24.3	2.5
1002	3-Iodo-4-aminobenzenesulfonic acid	0.05	0.13	11.5	14.9	...
46–91	N-(3,5-diiodobenzenesulfonyl)-glycine	0.21	0.42	11.1	9.3	1.2
46–27	Sodium N-(3,4,5-triiodobenzenesulfonyl)-glycinate	0.29	..	8.1	..	4.0
48–5	N-(4-hydroxy-3,5-diiodobenzenesulfonyl)-glycine	0.07	0.41	17.6	29.2	>5.0
46–31	Sodium N-(4-amino-3,5-diiodobenzenesulfonyl)-glycinate	0.05	0.10	25.0	48.1	>1.2
48–103	N-(4 amino-3,5-diiodobenzenesulfonyl)-glutamic acid	0	0.12	1.8	3.3	>5.0
49–188	7-Iodo-8-hydroxyquinoline-5-sulfonic acid	0	0.13	5.5	4.6	4.0

phthalein which also is in accordance with the clinical observations (9).

Table II also shows that the rpute of administration may greatly influence the level of excretion. When Diodrast® or Hippuran® were given intravenously, much greater amounts of iodine appeared in the urine during the three-hour period than when the drugs were given orally. Similar results were obtained with Skiodan®. However, although Neo-Iopax® falls into line with the other pyelographic compounds when given intravenously, only small amounts appeared in the urine after oral administration.

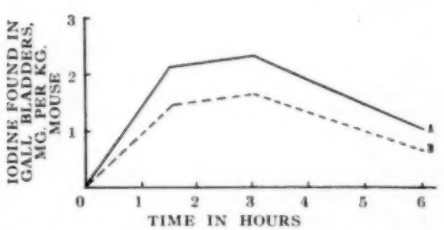

Fig. 1.—Iodine found in gall bladders at different time intervals. Curve *A* refers to an oral dosage level of 100 mg. of iodine (as Priodax®) per Kg. mouse. Curve *B* refers to an oral dosage level of 50 mg. of iodine (as Priodax®) per Kg. mouse.

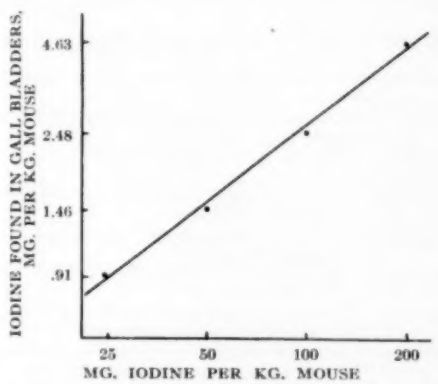

Fig. 2.—Dosage-response curve. Iodine found in gall bladders three hours after oral administration of different dosages of Priodax®. Both dosage and recovered iodine values are plotted logarithmically.

The study of some other available iodinated compounds has led to observations on the influence of structure on routes of excretion similar to those of Archer, *et al.* (5), Neuhaus, *et al.* (3), and Salter, *et al.* (6) who used other techniques to evaluate excretory channels. Following oral doses of iodinated compounds containing one or no benzene ring it was found in most cases that the predominant pathway for iodine excretion was in the urine. See Table III. These values were all obtained three hours after oral administration of the compounds in doses of 50 mg. and 100 mg. of iodine per Kg. weight. Since equal amounts of iodine were given to the mice, equal amounts should have been recovered at the excretory points unless the molecules were absorbed and/or metabolized by different actions in the body. This difference is especially notable with certain structures. For example only about 20% of the iodine from diiodotyrosine (49–187) was recovered in the urine and none in the gall bladder whereas over 50% of the iodine from compound 49–194 which does not contain the amino acid was recovered

TABLE IV.—EXCRETION OF IODINE IN GALL BLADDER AND URINE FOLLOWING ORAL ADMINISTRATION OF COMPOUNDS HAVING TWO OR MORE BENZENE RINGS

Compound Number	Compound	Recovered I_2 (mg./Kg. Body Weight) Gall Bladder 50 mg. I_2 per Kg. Dose	Gall Bladder 100 mg. I_2 per Kg. Dose	Urine 50 mg. I_2 per Kg. Dose	Urine 100 mg. I_2 per Kg. Dose	LD_{50} Oral, Gm./Kg.
47–33	α,α-dichloro-β,β-bis(*p*-iodophenyl)-ethylene	0	0	0.3	0.2	5.0
47–133	α,α-bis(*p*-iodophenyl)acetic acid	0.64	1.77	1.7	3.3	1.5
47–272	N-(4-hydroxy-3,5-diiodobenzoyl)-anthranilic acid	0.99	1.76	8.9	15.5	3.0
47–256	4-(4-hydroxy-3,5-diiodobenzamido)-benzoic acid	0.39	0.78	8.1	17.0	5.0
47–241	N-(4-amino-3,5-diiodobenzoyl)-anthranilic acid	0.08	0.44	0.1	0.5	5.0
47–240	4-(4-amino-3,5-diiodobenzamido)-benzoic acid	0.17	0.55	1.6	2.6	4.0
46–87	N-(3,5-diiodobenzenesulfonyl)-anthranilic acid	1.00	..	0.5	..	2.18
47–257	4-(3,5-diiodobenzenesulfonamido)-benzoic acid	1.31	2.38	4.2	7.9	5.0
46–134	Sodium N-(3,5-diiodobenzenesulfonyl)-sulfanilate	0.06	0.32	5.1	11.6	4.5
48–26	N-(4-hydroxy-3,5-diiodobenzenesulfonyl)-anthranilic acid	0.74	1.24	3.9	7.1	5.6
47–201	4-(4-hydroxy-3,5-diiodobenzenesulfonamido)-benzoic acid	0.18	0.24	2.2	11.0	5.0
46–28	N-3,5-diiodosulfanilylanthranilic acid	0.79	..	2.1	..	0.7
46–92	3-(3,5-diiodosulfanilamido)-benzoic acid	0.78	0.98	2.1	3.8	4.0
46–73	4-(3,5-diiodosulfanilamido)-benzoic acid	1.56	2.18	10.1	13.3	5.0
48–90	4-(3,5-diiodosulfanilamido)-phenylacetic acid	1.51	1.70	3.6	3.8	5.0
46–99	3-(3,5-diiodosulfanilamido)-benzenesulfonic acid	0.28	..	13.7	..	5.0
46–33	Sodium-N-3,5-diiodosulfanilylsulfanilate	0.17	..	3.7	..	1.2
46–186	N-(3,4,5-triiodobenzenesulfonyl)-anthranilic acid	1.42	2.11	1.9	3.4	5.0
47–255	3-(3,4,5-triiodobenzenesulfonamido)-benzoic acid	1.00	2.14	2.4	5.1	5.0
47–76	4-N-(3,4,5-triiodobenzenesulfonamido)-benzoic acid	0.50	1.76	2.6	4.6	5.0
46–129	3-(3,4,5-triiodobenzenesulfonamido)-benzenesulfonic acid	0.09	..	0.7	..	4.5
45–23	Sodium N-3,4,5-triiodobenzenesulfonylsulfanilate	0.29	..	1.5	..	4.0
46–110	4-N-(4-iodobenzenesulfonyl)-aminomethylbenzenesulfonic acid	0.13	..	12.5	..	5.0
46–96	4-N-(3,5-diiodosulfanilyl)-aminomethylbenzenesulfonic acid	0.51	..	2.8	..	5.0
46–94	4-N-(3,5-diiodosulfanilyl)-aminomethylbenzenesulfonamide	0.19	..	2.3	..	2.0
47–21	4-N-(3,4,5-triiodobenzenesulfonyl)-aminomethylbenzenesulfonic acid	0.30	0.31	0.5	0.8	4.0
46–122	4-N-(3,4,5-triiodobenzenesulfonyl)-aminomethylbenzenesulfonamide	0.22	..	1.9	..	5.0
46–34	Sodium 3,3′,5-triiododisulfanilamide	0.42	..	0.3	..	4.0
49–192	Diiodofluorescein	0.76	1.67	5.0	8.2	4.0
49–191	Tetraiodofluorescein (Iodeosin)	0.12	0.20	3.4	2.4	4.0

TABLE V.—RECOVERY OF IODINE FOLLOWING ORAL ADMINISTRATION OF TETRAIODOPHENOLPHTHALEIN AND IODOALPHIONIC ACID

	Dosage, Mg. I₂/Kg. Body Wt.	Administered Dose Recovered, %				
		Gall Bladder	Urine	Stomach	Small Intestine	Large Intestine
Unstarved Mice						
Iodoalphionic acid (Priodax®)	25	3.64	15.0	24.3	19.7	28.7
	50	2.92	9.6	28.1	22.4	18.1
	100	2.48	12.9	33.3	15.9	15.9
	200	2.31	15.6	31.7	20.6	8.8
Tetraiodophenolphthalein	50	2.12	1.1	32.6	23.2	11.6
	100	1.48	0.7	55.8	6.4	14.3
Starved Mice						
Iodoalphionic acid (Priodax®)	25	3.37	14.8	17.5	37.9	10.8
	50	2.29	10.4	16.5	35.8	20.2
	100	2.23	9.3	10.8	43.7	15.6
	200	1.78	22.4	4.4	34.0	18.2
Tetraidophenolphthalein	50	1.57	1.1	23.1	38.7	17.9
	100	1.49	0.8	46.2	25.5	6.1

in the urine. It may be seen that certain structures such as 2,3,5-triiodobenzoic acid (49–195); 3,5-diiodo-2-hydroxy benzoic acid (49–196); 2,3,4,5-tetraiodophthallic acid (49–186); 2,4,6-triiodophenoxy acetic acid (49–368); *n* - (3,5-diiodosulfanilyl) - glutamic acid (48–103); *n*-(3,5-diiodobenzenesulfonyl)-glycine (46–91); and 7-iodo, 8-hydroxyquinoline-5-sulfonic acid (49–188) were not conducive to rapid iodine excretion in the urine and only the diiodo (49–65) and the triiodo (49–195) benzoic acids and the triiodophenoxy acetic acid (49–368) increased the iodine content of the gall bladder to over 0.5 mg. per Kg. weight, but even this amount was not outstanding.

It is interesting to note that when the hydroxy group of the substituted 3,5-diiodo benzoate was shifted from the 2-position (49–196) to the 4-position (49–194) the iodine was readily excreted in the urine and the toxicity was reduced. This latter configuration is also seen in the thyroxine molecule.

When larger molecules containing two benzene nuclei were studied (Table IV) amounts of iodine approaching the level of 2.0 mg. per Kg. weight were often found in the gall bladder but the highest value found in the urine was only 17 mg. per Kg. weight. The sulfonamide linkage between the benzene rings appeared to enhance the iodine output in the gall bladder in certain instances but that in turn was dependent on certain hydrophylic groups. For example in the diiodo amino (46–73) and the diiodo (47–257) types the carboxy groups in the *para* position increased the amount of iodine in the gall bladder whereas in the diiodohydroxy analog (48–26) and in the triiodo type (48–186) the *ortho* carboxy group gave higher values.

When compounds of structure differing only in possessing either a benzoic acid or sulfonic acid group were compared the carboxy group enhanced excretion into the gall bladder. Compounds containing the $-SO_3H$ group tended to be found less in the gall bladder and sometimes less in the urine than the corresponding —COOH compounds. This was true of both *para* (47–257 *vs.* 46–134; 47–76 *vs.* 45–23; and 46–73 *vs.* 46–33) and *meta* positions (47–255 *vs.* 46–127; and 46–92 *vs.* 46–99). Predicting the excretory behavior of the compounds listed above appears to be extremely difficult. All of the compounds studied were relatively nontoxic as shown by the high LD_{50} values. These values did not appear to be influenced by routes or amounts of iodine excretion.

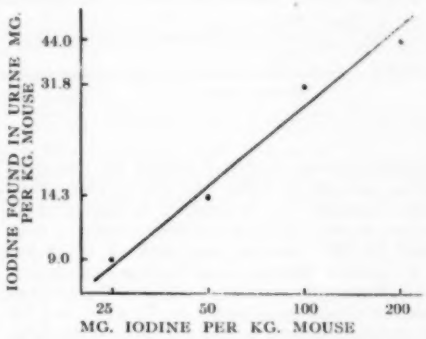

Fig. 3.—Dosage-response curve. Iodine found in three-hour urine collections after oral administration of different dosages of Diodrast®. Both dosage and recovered iodine values are plotted logarithmically.

It is reasonable to suppose that part of the differences in the amounts of excretion in bile and urine following the various orally administered compounds was due to variations in the rates of passage and absorption along the alimentary tract. Information on rates of passage was obtained by removing the gastrointestinal tract when the mice were killed and determining the iodine values in the different sections. For example, in one experiment in which a 100 mg. of iodine equivalent of iodoalphionic acid was compared with the same iodine equivalent of tetraiodophenolphthalein, it was found that only 33% of the former remained in the stomach as against a value of 56% for the latter three hours after administration as shown in Table V. In starved mice this difference was emphasized even more, the values being 11% and 46% respectively. Other regions of the tract were similarly investigated. Starvation did not noticeably affect excretion as it only tended to speed the material further along the gut. A few other representative

TABLE VI.—EXCRETION OF IODINE IN GALL BLADDER AND URINE AND RESIDUAL AMOUNTS IN GASTROINTESTINAL TRACT OF UNSTARVED MICE FOLLOWING ORAL DOSE OF 100 MG. IODINE PER KG. BODY WT.

Compound Number	Compound	Recovered Iodine (mg./Kg. Body Weight or %) Gall Bladder	Urine	Stomach	Small Intestine	Large Intestine
49–368	2,4,6-Triiodophenoxyacetic acid	0.89	3.5	11.6	15.1	15.6
49–195	2,3,5-Triiodobenzoic acid	1.49	6.0	6.5	11.7	5.2
		1.18	5.8	11.1	14.3	3.2
49–279	3,5-Diiodo-4-aminobenzoic acid	0.85	40.5	9.7	11.3	6.2
48–5	N-(4-hydroxy-3,5-diiodobenzenesulfonyl)-glycine	Trace	31.2	3.9	3.2	29.9
47–257	4-(3,5-diiodobenzenesulfonamido)-benzoic acid	1.83	10.3	5.8	20.9	26.5
46–134	Sodium N-(3,5-diiodobenzenesulfonyl)-sulfanilate	0.32	11.6	7.4	13.1	34.1
48–26	N-(4-hydroxy-3,5-diiodobenzenesulfonyl) anthranilic acid	1.28	10.3	2.3	9.2	55.0
47–201	4-(4-hydroxy-3,5-diiodobenzenesulfonamido)-benzoic acid	0.77	7.7	3.8	9.9	42.8
46–28	N-3,5-diiodosulfanilylanthranilic acid	2.69	19.1	9.7	22.6	9.7
46–73	4-(3,5-diiodosulfanilamido)-benzoic acid	2.19	24.7	10.3	29.8	7.9
46–186	N-(3,4,5-triiodobenzenesulfonyl)-anthranilic acid	2.16	4.6	25.8	21.6	15.7
47–255	3-(3,4,5-triiodobenzenesulfonamido)-benzoic acid	1.41	6.7	20.8	27.3	13.6
47–76	4-N-(3,4,5-triiodobenzenesulfonamido)-benzoic acid	1.77	11.3	18.0	22.4	16.4

compounds were similarly studied on unstarved animals as shown in Table VI. The reproducibility of the technique may be observed by examining the two sets of data obtained at different times for compound 49–195. Certain ones such as 48–26 and 47–201 passed through into the large intestine in much shorter time than the others but there was no correlation between excretion in the urine and bile and amounts present in the stomach, small intestine, and large intestine.

SUMMARY

1. A study of the excretion of iodinated compounds has been described which involves the chemical determination of iodine in the urine, bile, and gastrointestinal tract of the mouse.

2. Clinically used cholecystographic agents gave iodine values in the bile of over 1 mg. per Kg. body weight with low recovery of iodine in the urine. Amounts ranging up to 75% of the administered iodine were found in the urine following recognized pyelographic agents. Only traces of iodine were found in the gall bladder.

3. Results with other iodinated compounds studied indicated that prediction of excretory pathways was difficult. However, it appeared that most mono benzene types favored the urinary route while many of the compounds with two benzene rings enhanced the iodine content of the gall bladder, especially if certain other chemical groups were also present.

4. In the above method there appeared to be no correlation between rapidity of passage along the gastrointestinal tract and the channel of excretion.

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A New Series of Antifungal Compounds*

By E. B. HODGE, JOSEPHINE R. DAWKINS,† and ELEANORE KROPP‡

Compounds of the type ArCH(OR)CBr-(NO_2)R′ have been found to possess strong antifungal activity. The preparation, properties, and antifungal activities of a number of compounds of this formula are described. 2-Bromo-1-methoxy-2-nitro-1-phenylpropane has been further tested and ointments containing it have been prepared and submitted for clinical trial. Results from one of these trials have been published.

THE PREPARATION of five compounds of the type ArCH(OR)CX(NO_2)R′, where R and R′ are alkyl radicals and X is chlorine or bromine, has been described by Senkus (1). In a routine screening program one of the compounds, 2-bromo-1-methoxy-2-nitro-1-phenylpropane, (P–594) was found to be a very effective antifungal agent. Preparation of several analogous compounds indicated that for high antifungal activity the halogen should be bromine rather than chlorine. A number of compounds of this type were prepared and tested for antifungal activity. The compounds prepared, with their analyses, antifungal activities, and boiling or melting points, are shown in Table I which demonstrates that the presence of substituents on the aryl group in general lowered the antifungal activity.

The two compounds under No. 3 were separated by repeated crystallization from petroleum ether. They are believed to be the two diasteroisomeric mixtures which are possible for this compound. All of the other compounds also have two asymmetric carbon atoms and theoretically should exist in two such forms, but in no other case were the diasteroisomeric mixtures separated.

Because 2-bromo-1-methoxy-2-nitro-1-phenylpropane was about as effective as any of the other compounds, and also because this compound is perhaps more convenient to prepare than any other in the series, it was selected for further study. Additional antifungal data were obtained on this compound and these results are shown in Table II.

Formulated at one per cent in either a petrolatum or vanishing cream base this compound was

TABLE I.—COMPOUNDS OF THE TYPE ArCH(OR)CBr(NO_2)R′

No.	Ar	R	R′	M. P. or B. P., °C.	Yield, %	N, % Calc.	N, % Found	Br, % Calc.	Br, % Found	Min. Inhib. Concn. (μg./ml.) Against *Aspergillus niger*	Against *Trichophyton metagrophytes*	Against *Candida albicans*
1[a]	Phenyl	Me	Me	45–46	93	..	..	...	...	20	1	30
2	*p*-Chlorophenyl	Me	Me	96–98	88	4.54	4.34	25.90	25.85	100	5	>100
3	2,4-Dichlorophenyl	Me	Me	54–55.5	..	4.08	3.61	...	...	50	1	5
				92–94.5	..	4.08	3.77	...	...	5	1	1
4	2,4-Dichlorophenyl	Me	Et	130–140[b]	..	3.92	3.70	22.38	22.44	50	5	>100
5	*p*-Anisyl	Me	Et	144–147[b]	51	4.40	4.36	25.11	25.63	100	5	>100
6	*p*-Anisyl	Me	Me	74–75	55	4.61	4.41	26.28	27.36	<1	50	100
7	*p*-Chlorophenyl	Me	*n*-Propyl	107–108	45	4.16	4.23	...	...	10	10	>100
8	Phenyl	2-Hydroxyethyl	Me	...	79	4.61	4.52	...	...	100	5	100
9	Furyl	Me	Me	92–97[b]	23	5.30	5.59	...	...	10	1	25
10	*o*-Chlorophenyl	Me	Me	120[b]	57	4.54	4.44	...	...	25	<1	50
11	*o*-Chlorophenyl	Et	Me	122[b]	61	4.34	3.96	...	...	25	5	25
12	*o*-Chlorophenyl	Et	Et	128[b]	28	4.16	3.93	...	...	10	5	100
13	*o*-Chlorophenyl	Me	Et	128[b]	38	4.34	3.97	...	...	10	5	>100
14	*p*-Chlorophenyl	Me	Et	104.5–106	44	4.34	4.03	...	...	25	<1	100
15	3,4-Dichlorophenyl	Me	Me	67–70	66	4.08	3.69	...	...	25	5	50
16	Phenyl	Et	Et	113–115[b]	45	4.63	4.85	...	...	<1	<1	100

[a] Prepared by Senkus.
[b] B. p. at 1–2 mm. of mercury.

* Received November 5, 1953, from the Pharmaceutical Research Division of Commercial Solvents Corporation, Terre Haute, Ind.
The authors are indebted to G. L. Lafferty for formulation work, and to Dr. J. N. Spencer for pharmacological testing. Analyses were performed under the direction of Dr. J. A. Riddick.
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found to cause little or no irritation when applied daily for seven days to the intact skin of twelve rabbits. Preliminary clinical trials of this compound in one per cent concentration in a vanishing cream base have been carried out by several physicians and the results of one of them have been published (2). The conclusions reached in the cited reference were as follows: "This compound possesses low toxicity, little irritancy, an apparent low sensitizing index and a high degree of efficiency in the treatment of mycotic lesions of glabrous skin. It is considered of particular value in the treatment of infections caused by *T. rubrum*."

EXPERIMENTAL

Preparation of Compounds.—The preparation of all of these compounds was carried out essentially as described in reference (1), examples II and IV.

The preparation of the two forms of 2-bromo-1-(2,4-dichlorophenyl)-1-methoxy-2-nitropropane was carried out as follows: A mixture of 46.4 Gm. of 1-(2,4-dichlorophenyl)-2-nitro-1-propene, 200 cc. of methanol, 75 cc. of water, and 10 Gm. of sodium hydroxide was stirred to give an almost clear solution and then filtered. The filtrate was stirred and cooled in an ice bath and 10.2 cc. of bromine was added dropwise at not over 20°, causing the formation of an oil which slowly solidified. This was filtered to give 60.4 Gm. of a solid, melting from 55–70°. This was recrystallized twice from petroleum ether and the filtrates were combined. From them was isolated 17 Gm. of the lower melting product. The precipitate from the second crystallization was recrystallized twice more to give 12.6 Gm. of the higher melting product.

TABLE II.—ACTIVITY OF P-594 AGAINST YEASTS AND FUNGI

Culture	Minimum Inhibitory Concentration (μg./ml.)
M. audouini, 10216	1
S. schenckii, 10212	25–50
E. floccosum, 10227	1
T. mentagrophytes, 10270	1–5
T. rubrum, 10218	1–5
B. dermatitidis, 10225	1
A. fumigatus, 6285	5–10
C. albicans	10–25
A. boris, 10048	10–25
Schizosacharomyces pombe ATCC, 2476	5–10
Endomyces magnusii ATCC, 2105	5–10
Saccharomyces lactis ATCC, 8635	10–25
Saccharomyces cerevisiae Sc31	10–25
Zygosaccharomyces mandshuricus NRRL, Y-301	10–25
Torulaspora rosei NRRL, Y-1567	10–25
Debaryomyces matruchoti NRRL, Y-833	5–10
Endomyces jaronensis NRRL, Y-1483	1–5
Mycoderma cererusuae NRRL, Y-932	5–10
Kloeckera brevis NRRL, Y-915	1–5
Brettanomyces anamalus NRRL, Y-1415	5–10
Candida guilliermondia NRRL, Y-324	25–50
Rhodotorula mucilaginosa var. *ruhrorugosa* ATCC, 4559	50–100
Hansenula saturnos NRRL, Y-12	10–25
Candida keusei NRRL, Y-301	10–25
Aspergillus niger	10–20

Antifungal Testing.—Stock solutions of the compounds being tested were prepared at 1% concentration using a suitable solvent. In initial testing only the concentration 100 μg./ml. was used. This was obtained by diluting the stock solution 1:10 with water and adding a 2-cc. aliquot to 18 cc. of Sabouraud's agar with 0.5% Difco Malt extract. The agar and agent were then steamed for thirty to forty-five minutes. Plates were poured, allowed to solidify, and streaked with suspensions of cells contained in a 7-mm. bacteriological loop. The suspension was prepared from a fresh slope washed into saline and should show about 50% of the light transmission of a saline control using 15- to 16-mm. (i. d.) tubes and a red filter. The fungi used in the initial series of tests were *Aspergillus niger*, *Trichophyton mentagrophytes*, and *Candida albicans*. Plates were checked after five days at 25°. If no growth occurred, the process was repeated at lower concentrations.

SUMMARY

1. A class of antifungal compounds of the type $ArCH(OR)CBr(NO_2)R'$ has been studied and sixteen members of it have been prepared.

2. The compound 2-bromo-1-methoxy-2-nitro-1-phenylpropane has been found to be fungistatic at low concentrations toward a number of yeasts and fungi.

3. This compound, formulated in an ointment at one per cent concentration, has been submitted for clinical trial.

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Steroidal Sapogenins*

XII. Survey of Plants for Steroidal Sapogenins and Other Constituents

By MONROE E. WALL,† C. ROLAND EDDY,† J. J. WILLAMAN,† D. S. CORRELL,‡ B. G. SCHUBERT,‡ and H. S. GENTRY‡

This is a report of the chemical examination of the second 1,000 samples received in a survey of plants for steroidal sapogenins. Data are given for 997 samples, representing 598 identified species, 201 unidentified lots, 392 genera, and 129 families. There is no previously published chemical information on about 60 per cent of the species examined. Quantitative data are given for the occurrence of 11 steroidal sapogenins. These were found almost exclusively in *Yucca, Agave,* and *Dioscorea.* A new sapogenin, named markogenin, was found in *Y. schidigera.* Qualitative data are given for the occurrence of the following groups of constituents: flavonols, alkaloids, tannins, and unsaturated sterols.

PAPER NUMBER VII in this series covered the survey of the first 1,000 samples (1). The present report is a continuation of it and covers the second 1,000 samples. The objective and assay procedures were described in detail in the former report and are not repeated here.

The data include the kinds and amounts of steroidal sapogenins found, and qualitative tests for saponins, flavonols, alkaloids, tannins, and unsaturated sterols. There are 997 samples, representing 598 identified species, 201 unidentified lots, 392 genera, and 129 families. There is no published chemical information on about 60 per cent of the species examined.

PROCUREMENT

The material represents a general sampling of the plant kingdom, as evidenced by the 364 genera in 107 families not included in the first thousand. Besides this continued general survey of the plant kingdom, an extensive collection was made of species in the genera *Dioscorea* and *Agave.*

Collections of *Agave* were obtained primarily by H. S. Gentry in Mexico and the southwestern United States and from the living collection maintained at the Huntington Botanical Garden in San Marino, Calif. He also collected numerous samples of *Dioscorea* in Mexico.

General collections were obtained not only from the above-mentioned areas but also from several cooperators in the southeastern United States, Brazil, Colombia, Okinawa, and elsewhere. Approximately half the collections, however, were procured from permanent stock maintained at the Division of Plant Exploration and Introduction gardens at Coconut Grove, Fla.; Glenndale, Md.; and Chico, Calif.

RESULTS

A table has been prepared giving the data for each sample—its origin and identification, results of the hemolysis tests, kinds and amounts of steroidal sapogenins found, and qualitative findings for flavonols, alkaloids, tannins, and sterols. Because of space limitations, this table cannot be given here. It has, however, been prepared in processed form as AIC-367 and may be obtained on request from the U. S. Department of Agriculture, Eastern Regional Research Laboratory, Philadelphia 18, Pa.

In Table I the steroid data are rearranged to show their occurrence by species.

A positive hemolysis test for saponin was obtained in about 50% of the species. Steroidal sapogenins were found, however, almost exclusively in *Agave, Yucca,* and *Dioscorea,* the exceptions being *Manfreda* (Amaryllidaceae) and *Cordyline* (Liliaceae).

Four rarer steroidal sapogenins—kammogenin, samogenin, yammogenin, and yuccagenin—found in the first series were not found in the present one. However, a hitherto unknown one was found in the by-product leaf powder from the production of fiber from *Yucca schidigera* (sample 1686-0). It was named markogenin (2), spirostan-2ξ,3β-diol, a 2,3-dihydroxy analog of sarsasapogenin.

As stated in the previous report, the nature of the sapogenins found in plants is largely species specific. *Agave* species for the most part produce compounds with a *trans* ring A/B and with *iso* ring configuration at carbon 22. Depending on a number of environmental factors, tigogenin, hecogenin, manogenin, gitogenin, and occasionally chlorogenin are found singly or in combination. In this genus,

* Received January 8, 1954, from the Agricultural Research Service, U. S. Dept. of Agriculture.

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In order to expedite the examination of such a large number of plant materials, groups of Laboratory workers were organized, each group handling certain phases of the whole procedure. The authors gratefully acknowledge the work of J. W. Garvin, Walter Rumph, R. A. Pierce, H. M. Neilson, G. H. Eppley, Theodore Perlstein, H. E. Kenney, Arthur Finchler, H. W. Jones, M. L. McClennan, Samuel Serota, R. F. Mininger, H. I. Sinnamon, A. E. Jones, C. S. Fenske, M. K. Scott, M. A. Morris, and J. R. Necho.

In the procurement phase, in addition to those mentioned above, the authors wish to thank the following for their cooperation: W. H. Hodge, in Arizona; W. H. Duncan, in Georgia; R. K. Godfrey, in North Carolina; H. Hurlimann in New Caledonia; Egbert Walker, in Okinawa; C. L. Gilly in Mexico; Mrs. S. Stafford, in California.

TABLE I.—OCCURRENCE OF THE VARIOUS SAPOGENINS BY SPECIES

Species	No. of Samples	Genin Content, —M. F. B., %— Min.	Max.
Chlorogenin			
Agave brandegeei	1	..	Trace
Agave schidigera	1	..	Trace
Agave sp.	1	..	0.1
Manfreda sp.	1	..	0.5
Yucca angustissima	1	..	Trace
9-Dehydrohecogenin			
Agave aurea	1	..	0.05
Agave cerulata	2	..	Trace
Agave nelsonii	5	Trace	0.2
9-Dehydromanogenin			
Agave aurea	1	..	Trace
Agave cerulata	2	Trace	0.5
Agave goldmaniana	1	..	Trace
Agave nelsonii	5	Trace	0.5
Agave promontorii	1	..	Trace
Agave vexans	1	..	Trace
Agave virginica	1	..	0.1
Diosgenin			
Dioscorea bartlettii	1	..	Trace
Dioscorea composita	1	..	3.8
Dioscorea glauca	1	..	0.2
Dioscorea villosa	5	0.5	1.2
Dioscorea sp.	3	1.0	2.0
Gitogenin			
Agave cerulata	1	..	Trace
Agave goldmaniana	1	..	Trace
Agave roseana	2	..	Trace
Agave schidigera	1	..	Trace
Agave aff. *schidigera*	1	..	0.3
Agave toumeyana	1	..	Trace
Agave virginica	1	..	0.8
Agave sp.	2	Trace	0.2
Manfreda sp.	2	0.7	1.0
Yucca peninsularis	2	Trace	0.3
Yucca whipplei	2	Trace	0.1
Hecogenin			
Agave atrovirens	1	..	0.8
Agave aurea	3	0.1	0.7
Agave brandegeei	3	0.1	0.6
Agave cerulata	3	Trace	0.2
Agave chrysantha	1	..	0.2
Agave datylio	1	..	0.5
Agave deserti	1	..	0.4
Agave expansa	1	..	0.1
Agave goldmaniana	1	..	0.1
Agave nelsonii	5	0.1	0.7
Agave parryi	1	..	0.2
Agave promontorii	2	0.2	0.7
Agave roseana	3	0.1	0.3
Agave shawii	1	..	0.2
Agave sullivanii	1	..	0.1
Agave toumeyana	1	..	0.6
Agave vexans	1	..	0.2
Agave sp.	3	0.2	0.4
Yucca peninsularis	1	..	0.1
Manogenin			
Agave aurea	1	..	Trace
Agave cerulata	2	..	Trace
Agave chrysantha	1	..	Trace
Agave goldmaniana	2	..	Trace
Agave nelsonii	4	Trace	0.7
Agave promontorii	1	..	Trace
Agave roseana	2	..	Trace
Agave shawii	1	..	0.3
Agave toumeyana	1	..	0.3
Agave vexans	1	..	0.2
Agave virginica	1	..	0.2
Agave sp.	2	..	Trace
Markogenin			
Yucca schidigera	1	..	0.2
Sarsasapogenin			
Cordyline neo-caledonica	1	..	0.5
Dioscorea bartlettii	1	..	Trace
Yucca angustissima	1	..	0.2
Yucca arizonica	1	..	0.3
Yucca baccata	2	0.5	0.8
Yucca elata	2	0.5	1.3
Yucca faxoniana	1	..	0.2
Yucca schidigera	1	..	2.9
Yucca sp.	2	0.8	0.9
Smilagenin			
Agave carchariodonta	1	..	0.4
Agave difformis	1	..	0.4
Agave goldmaniana	1	..	0.3
Agave lophantha	1	..	1.0
Agave sp.	1	..	1.0
Yucca sp.	1	..	1.5
Tigogenin			
Agave asperrima	1	..	0.4
Agave brandegeei	3	0.1	0.3
Agave cerulata	2	..	Trace
Agave nelsonii	1	..	0.1
Agave promontorii	1	..	0.1
Agave roseana	4	0.1	0.6
Agave schidigera	1	..	0.1
Agave aff. *schidigera*	1	..	0.7
Agave sullivani	1	..	Trace
Agave sp.	4	Trace	1.6
Manfreda sp.	1	..	0.6
Yucca faxoniana	1	..	0.3
Yucca peninsularis	3	0.4	0.9
Yucca whipplei	2	0.3	0.6

hecogenin is probably found most frequently, although rarely alone. A few agaves produce smilagenin with the *cis* ring A/B and *iso* carbon 22 configuration. In these cases, the sapogenin is usually not accompanied by isomeric or modified forms. Sarsasapogenin with a *cis* ring A/B and normal carbon 22 configuration was again found as the sole or predominant constituent of a number of western yuccas. However, coastal yuccas, both from the Southwest and Southeast, produce tigogenin and gitogenin. As stated in the previous paper, *Dioscorea* species produce diosgenin as the sole or predominant steroid.

The evidence presented in this and the preceding report, with few exceptions, permits the following generalization. Sapogenin configuration at carbon 5 and carbon 22 is genus and species specific and cannot be altered by the plant or by environmental conditions. Ketonic and hydroxyl groups, however, can be added or removed by numerous species, resulting in the formation of mixtures of sapogenins.

None of the first 1,000 samples gave a triple test for flavonols. In the present list, however, three were found—leaves of *Diervilla richesse* and *Lonicera* sp. in the Caprifoliaceae and leaves and fruit of *Garcinia spicata* in the Hypericaceae. Krewson, *et al.* (4), point out that of 27 species of *Eucalyptus*

examined for rutin, only two contained it. We obtained a test for flavonol in *E. staigeriana* (Myrtaceae), not one of the species hitherto examined. It remains to determine whether this flavonol is rutin.

No test for alkaloid was obtained in 141 samples of *Dioscorea* tubers—18 identified species and 85 lots of unidentified—native to North, Central, and South America, and to the West Indies. In 42 samples native to the rest of the world, alkaloids were found in seven species. This, together with other published evidence, has led to the generalization that the alkaloids probably do not occur in *Dioscorea* in the Western Hemisphere but that they do occur in some Old World species (3). No sapogenins were found in *Dioscorea*-containing alkaloids. Of the species which gave a two- or three-plus test for alkaloids, the following are the first on record: *Lycoris squamigera* (Amaryllidaceae); *Buxus harlandi* (Buxaceae); *Lophocereus schottii* (Cactaceae); *Dioscorea dumetorum* (Dioscoreaceae); *Adenocarpus foliosus, Cassia brasieliensis, C. emarginata, C. excelsa, Peltogyne nitens, Pithecellobium flexicaula* (Leguminosae); *Neillia longiracemosa* (Rosaceae); *Cephalotaxus henryi* (Taxaceae).

As to tannins, the samples for most families are too few for generalization. However, tannins appear to occur frequently in Anacardiaceae, Caprifoliaceae, Ericaceae, Leguminosae, Myrtaceae, and Rosaceae.

As in the first series, unsaturated sterols were generally abundant and frequent.

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The Blood Pressure Lowering Ability of Some Fractions of *Rauwolfia serpentina* Benth.*

By CARL A. SCHLAGEL† and JOHN W. NELSON‡

A study has been made of the antihypertensive activity of nine samples from five different varieties of *Rauwolfia serpentina*. A difference in potency among the different samples and varieties, and the variation in response between animals demonstrates the need for an assay procedure for purposes of standardization of the activity. The prolonged action of certain of the preparations tested and a high therapeutic index suggest that the drug is worthy of further chemical and pharmacological study.

THE NEED for additional drugs for the control of hypertension has received considerable attention in the recent literature (1, 2). This need arises from the apparent nonspecific nature of hypertension in which more than one etiologic agent may presumably be responsible. Some of the desirable properties which any new hypotensive agent should possess appear to be: prolonged action, freedom from side effects and the development of tolerance, high therapeutic index, and capability of lowering the blood pressure without precipitating dangerous hypotensive crises. The possibility that the activity of *Rauwolfia serpentina* may be characterized by several of these properties is indicated in the Indian and European literature which has been forthcoming since Sen and Bose first revealed the hypotensive effect of this plant in 1931 (3). An excellent review of this literature as well as an account of the chemistry which was employed in preparation of the fractions tested in the present study has been prepared by Holt and Costello and can be found in a previous issue of THIS JOURNAL (4).

The present investigation was begun after preliminary experiments conducted in this laboratory by Nelson in 1951 confirmed that *Rauwolfia serpentina* did possess hypotensive activity (5). This is a continuing study, and the present report deals with the determination of the relative antihypertensive activity of the alcoholic extracts of several commercial varieties as well as some alkaloidal fractions thereof.

EXPERIMENTAL

The laboratory animals employed were normotensive, mongrel dogs of mixed sex and variable weights. They were anesthetized by intraperitoneal injection with 30 mg./Kg. or more of veterinarian sodium pentobarbital. The right carotid blood pressure was recorded with a mercury manometer. The trachea was cannulated and respiration recorded with a membrane tambour. In the majority of ex-

* Received April 12, 1954, from The Ohio State University College of Pharmacy, Columbus, Ohio.
Abstracted in part from a thesis submitted to the Graduate School of The Ohio State University by Carl A. Schlagel in partial fulfillment of the requirements for the degree of Master of Science.
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TABLE I.—RESPONSE OF TWO DOGS TO THE ALCOHOLIC EXTRACTS *B* (BIHAR), *K* (DEHRA DUN), *L*-7784 (HIMALAYAN), AND *P* (HIMALAYAN)

Animal No.	Weight, Kg.	Alcoholic Extracts, Each cc. = 1 Gm. Crude Drug	Dose, cc.	Blood Pressure, mm. Hg: Pre-injection Level	Blood Pressure, mm. Hg: Level at Nadir	Drop, %
1	11	*B*	0.25	120	110	8
		B	0.50	120	96	20
		B	0.75	120	92	23
		B	1.00	120	72	40
		K	0.25	95	40	58
		K	0.50	72	35	51
		K	0.75	62	30	52
2	11.4	*L*	0.25	155	135	13
		L	0.50	150	105	30
		L	0.75	150	115	23
		L	1.00	125	85	32
		P	0.25	105	55	48
		P	0.50	100	50	50
		P	0.75	80	45	44

periments, the drugs were administered intravenously via a cannulated femoral vein which was connected by rubber tubing to a graduated buret. Each dose of drug was washed in with 5 cc. of normal saline.

The blood pressure changes were measured from the pre-injection level to the nadir of the blood pressure tracings and computed as per cent fall in terms of the pre-injection level. Before any drugs were injected, a tracing of the normal, constant blood pressure level was recorded. From time to time an equivalent volume of ethanol, which was the vehicle for most of the preparations unless otherwise specified, was administered to assure that it did not hinder or contribute adversely to the hypotensive response. It is recognized that ethanol is not completely free from physiological effects, but it was used in lieu of other solvents because of several technical difficulties.

The sequence of dosage of various fractions as well as the time between injections was largely dictated by the activity of the various fractions or the response of the animals. In most cases one hour or more was necessary between doses. In all cases unless otherwise specified, each cc. of the alcoholic extracts and alkaloidal preparations was equivalent to 1 Gm. of crude drug.

PROCEDURE AND RESULTS

Alcoholic Extracts.—The sequence of doses and the hypotensive responses of four dogs which were administered the alcoholic extracts of four commercial varieties of *Rauwolfia serpentina* are presented in Tables I and II. According to the place of origin or source of supply, these samples were designated as *K* (Dehra Dun), *B* (Bihar), *P* (Himalayan), and *L*-7784 (Himalayan). In two other dogs, one of which received four doses of *K* followed by four doses of *B*, and the other which was given two doses of sample *P*, the responses were similar in most every respect with those recorded in Tables I and II.

TABLE II.—RESPONSE OF TWO DOGS TO THE ALCOHOLIC EXTRACTS *B* (BIHAR), *K* (DEHRA DUN), *L*-7784 (HIMALAYAN), AND *P* (HIMALAYAN)

Animal No.	Weight, Kg.	Alcoholic Extracts, Each cc. = 1 Gm. Crude Drug	Dose, cc.	Blood Pressure, mm. Hg: Pre-injection Level	Blood Pressure, mm. Hg: Level at Nadir	Drop, %
3	5.0	*B*	0.50	130	80	39
		K	0.50	140	55	61
		B	0.50	105	65	38
		K	0.50	105	20	81
		L	0.50	100	65	35
		P	0.50	100	45	55
		L	0.50	80	65	19
		P	0.50	80	55	31
4	5.4	*L*	0.50	150	130	13
		P	0.50	150	60	60
		L	0.50	100	70	30
		P	0.50	100	40	60
		L	0.50	90	40	55
		P	0.50	80	25	69

For purposes of comparison, alternating doses of *K* and *P* which were the most potent samples and a standardized tincture of *Veratrum viride* were administered to a 14.0-Kg. dog. The *Veratrum viride* in doses of 0.21 cc./dog (1.0 cc. represents 0.1 Gm. of crude drug) reduced the pressure approximately 33%. Both *Rauwolfia* samples in doses of 0.25 cc./dog (1.0 cc. represents 1.0 Gm. crude drug) reduced the pressure to the same or slightly greater extent. In terms of crude drug, the *Rauwolfia* samples were roughly one-tenth as active as the *Veratrum viride*.

At a later date, two additional *Rauwolfia serpentina* samples (*BK*-3690-Himalayan and *BK*-3707-Coastal Plain) were received and tested as the alcoholic extracts. When given alone to two dogs, they appeared to be less active than the potent *K* (Dehra Dun) and *P* (Himalayan) samples. This order of potency was more or less verified from the response of a 17.5-Kg. dog which received alternating doses of these two new samples along with sample *K*. A total of three 0.5-cc. doses of sample *BK*-3690 (Himalayan), two of *BK*-3707 (Coastal Plain), and two of the Dehra Dun sample produced reductions in pressure of 24, 42, and 28%; 32 and 24%, and 74 and 37%, respectively.

Comparative potency tests in another dog weighing 12.7 Kg. revealed that these two samples were also less potent than still another drug sample designated as *L*-8808 (Dehra Dun). In 1.0-cc. doses, the *BK*-3690 (Himalayan), *BK*-3707 (Coastal Plain), *L*-7784 (Himalayan), and *L*-8808 (Dehra Dun) reduced the blood pressure 25, 26, 26, and 48%, respectively.

Administration of 1.0-cc. doses of sample *L*-8808 (Dehra Dun) alone to a 10.0-Kg. dog produced falls in pressure of 54 and 78%. In another animal weighing 8.6 Kg., three successive 0.75 cc. doses of this same extract lowered the blood pressure 71, 58, and 64%.

Another alcoholic extract which was obtained from *RKP*-207-222 (Malabar) material and which was available in some quantity was given to five dogs. Interest in this drug sample was soon abandoned when doses ranging from 0.3 cc./Kg. to 0.18 cc./Kg. produced violently oscillating blood pressure tracings which were erratic and inconsistent. Other side effects caused by the drug were Biot's breathing and/or bouts of severe hyperpnea, and periods of recovery from anesthesia with generalized struggling. The explanation for this erratic action became apparent when a subsequently submitted pharmacognostical examination showed that the Malabar and Coastal Plain varieties were moderately to grossly adulterated with another species of *Rauwolfia* while all the others examined were authentic *Rauwolfia serpentina* (4). Chemical examination also revealed considerable differences in the proportions of individual alkaloids between the adulterated and authentic species (4). Comparison tests in two dogs showed that this sample was less potent than the *L*-8808 (Dehra Dun) variety.

As the vagal effects oftentimes render the measurement of the mean arterial pressure both difficult and inconsistent, a preliminary site of action study was conducted to determine if the vagi could be eliminated without altering the typical hypotensive response produced by *Rauwolfia serpentina* preparations. Four doses of the alcoholic extract were administered at hourly intervals both before and after sectioning of each of the vagi and before and after occluding the left carotid artery (right carotid previously occluded to record the blood pressure). Before any doses were injected, the carotid reflex response in each dog was checked to assure that the animal responded to carotid clamping. After each vagus was cut, a four- to five-minute delay was permitted before injection of the drugs to permit the blood pressure to resume a more or less constant level. In contrast, after the carotid was occluded, the extracts were injected at the peak of the rise in blood pressure. The tabulated results of this experiment are presented in Table III. The heart rate which was counted in two dogs decreased from 10 to 30 beats per minute immediately after injection of all doses of the drug, and then increased to its pre-injection norm in a manner parallel to the return to normal of the blood pressure. This same effect upon the heart rate was manifest in many of the other experiments in which the vagi were intact.

Response to Oral Administration.—The alcoholic extracts *K* (Dehra Dun), *P* (Himalayan), *L*-7784 (Himalayan), and *L*-8808 (Dehra Dun), as well as two fractions each containing the crude serpentine group of alkaloids were given orally via stomach tube or injected directly into the exposed duodenum just caudad to the pyloric sphincter. All of these preparations had previously demonstrated excellent blood pressure lowering activity. In a 15-Kg. dog, an oral dose of 2.5 cc. of sample *K* produced a slight rise in the pressure over a period of several hours. When 5.0 cc. of sample *P* was injected into the duodenum, the blood pressure dropped slowly and progressively from 145 to 85 mm. Hg, or 41%. After four hours, 6 mg./Kg. of the serpentine alkaloids also given intraduodenally caused a further drop in pressure from 95 to 75 mm. Hg.

In another dog weighing 14 Kg., 3.0 cc. of extract *K* given orally reduced the carotid pressure from 178 to 145 mm. Hg in one hour. The pressure remained at this level for three hours whereupon 3.0 cc. of extract *P* was given orally. A further fall in pressure from 145 to 138 mm. Hg, or a drop of 5% was recorded. Oral administration of the *L*-7784 and *L*-8808 alcoholic extracts and another serpentine

TABLE III.—EFFECT OF SECTIONING OF THE VAGI AND OCCLUSION OF THE LEFT CAROTID ARTERY ON THE HYPOTENSIVE ACTIVITY OF A HIMALAYAN (*L*-7784) ALCOHOLIC EXTRACT

Animal Preparation	Dose, cc./Kg.	Fall in Blood Pressure, %				
		Animal, No. 1	Animal No. 2	Animal, No. 3	Animal, No. 4	Av.
Normal	0.05	41	11	17	21	23
After right vagus cut	0.05	41	15	12	27	24
After left vagus cut	0.05	42	14	32	33	30
After left carotid occluded	0.05	43	35	58	35	43

TABLE IV.—HYPOTENSIVE ACTIVITY OF 2.0 MG./KG. DOSES OF STRONGLY BASIC ALKALOIDS SEPARATED FROM A MALABAR DRUG SAMPLE

Animal No.	Alkaloidal Preparation	Blood Pressure, mm. Hg: Pre-Injection Level	Blood Pressure, mm. Hg: Pressure at Nadir	Drop, %
1	Crude serpentine group (*DA*)	124	88	29
	Pure serpentine alkaloid (*DB*)	116	74	36
	Crude serpentine group (*DA*)	106	70	34
	Impure serpentinine alkaloid (*DC*)	110	66	40
	Crude serpentine group (*DA*)	105	70	33
2	Impure serpentinine alkaloid (*DC*)	112	54	52
	Pure serpentine alkaloid (*DB*)	88	58	34
	Crude serpentine group (*DA*)	83	52	37
3	Impure serpentinine alkaloid (*DC*)	120	96	24
	Crude serpentine group (*DA*)	120	88	27
	Pure serpentine alkaloid (*DC*)	114	76	33
	Impure serpentinine alkaloid (*DC*)	110	72	35
	Crude serpentine group (*DA*)	108	60	44

alkaloidal fraction had no effect whatsoever in a third animal. Defecation with loose, watery stools occurred after administration of sample *P* in the first dog and samples *P* and *K* in the second animal.

Alkaloidal Fractions.—A total of ten dogs was utilized in determining the antihypertensive activity of the following preparations which were separated from the *K* (Dehra Dun) sample:

(*a*) The crude total ajmaline and crude total serpentine alkaloids separated both by the slightly modified Siddiqui method (6) and by the method of Schlittler and Schwarz (7).

(*b*) Ajmaline, ajmalicine, and serpentine alkaloids.

(*c*) Ajmaline residue (residue from total ajmaline fraction after removal of the ajmaline, ajmalinine, and ajmalicine).

(*d*) Serpentine residue (residue after removal of the serpentine by crystallization).

The hypotensive responses in these animals revealed that the pure serpentine alkaloid was always more potent and longer acting than the other individual ajmaline alkaloids, and that the total serpentine alkaloids were more potent than the total ajmaline group. Both alkaloidal residues were quite active, the serpentine residue being slightly the most potent preparation tested. The high potency of the ajmaline residue can probably be accounted for by the fact that subsequent chemical experience with the alkaloids revealed that approximately 10% of the more potent serpentine group of alkaloids was mistakingly retained in the crude ajmaline group during the course of the chemical separation. The portion so retained was the relatively weakly basic portion which was precipitated by ammonia from the more strongly basic serpentine group of alkaloids.

Because the serpentine alkaloids consistently lowered the pressure to a greater extent and for a longer period of time, the potency of two additional crude total serpentine alkaloidal preparations, one from the previously tested *L*-7784 (Himalayan) variety, and the other from a new but adulterated *BK*-3490 (Malabar) sample was ascertained. Three 2.0 mg./Kg. doses of each of these fractions given alternately to one dog consistently lowered the blood pressure between 36 and 44%. When the above Malabar preparation containing the crude total serpentine alkaloids (Code *DA*) was further fractionated into a pure serpentine alkaloid (Code *DB*) and an impure serpentinine fraction (Code *DC*) and all three preparations tested in the same animals, the activity was for the most part evenly divided as shown in Table IV. The exact nature and character of this impure serpentine fraction has not yet been elucidated, but two doses of a pure serpentinine alkaloid indicate that it is only moderately potent compared to pure serpentine.

Further work with the alkaloids involved a comparison of the alcoholic extract of the above Malabar sample with the total alkaloids as separated by the British Pharmaceutical Codex (1949) method from this alcoholic extract. In doses representing 1.0 Gm. of crude drug/8 Kg. dog, the alcoholic extract reduced the blood pressure 47% and the total alkaloids 21% in the same animal. In another dog, the reductions were 26% and 13%, respectively. Similar determinations involving other drug varieties discloses the same but less pronounced potency discrepancies between the alcoholic extract and the total alkaloids derived therefrom by the B. P. C. method.

General Responses.—The type of antihypertensive response produced by *Rauwolfia serpentina* was generally similar for equipotent doses of all of the preparations tested. Following the more potent doses, the pressure fell sharply to nadir, rose slightly, fell again to various but frequently quite low levels, and then slowly and progressively ascended to a value 10 to 20 mm. Hg below the pre-injection level. The climb toward normal usually required from one to three hours. Following small doses of the less potent samples and preparations, the responses were of short duration and the pressure usually returned to normal. With the exception of the adulterated varieties, the respiration was not adversely affected. Several of the alkaloidal fractions, however, caused momentary apnea immediately after their injection, and slight respiratory depression throughout the experiment.

Defecation was the only other observable untoward side effect. It appears to be correlated with the degree of hypotensive response as the three most potent alcoholic extracts were the chief offenders. They always produced defecation unless the bowels had been previously evacuated by other doses of drug. The moderately potent alcoholic extracts and the alkaloidal fractions caused defecation, but

did so rarely and only when large doses were employed.

Another fact worthy of note was the variability in degree of hypotensive response of different dogs to equivalent doses of the same preparation. Between dogs, the per cent reduction in pressure often varied as much as 100%. Within the same animal, the responses were not as variable; however, as a general rule the per cent reduction in pressure based on the pre-injection level became greater as the experiment progressed. This naturally followed from the fact that as the pre-injection level became lower and lower for each succeeding dose in relation to approximately the same mm. Hg drop in pressure, the greater was the calculated per cent fall in pressure.

Acute Toxicity.—The acute toxicity of the alcoholic extract *L*-8808 (Dehra Dun) was determined by i. v. injection in healthy, albino, Swiss-strain mice. In order to obtain the proper injection volume, the alcoholic extract was diluted 1:10 with a 0.5% solution of sodium carboxymethylcellulose medium viscosity.[1] Five groups of animals consisting of 12 mice each received graded doses of the drug dilution, and two groups of six more served as controls. Other usual requirements for acute toxicity studies were satisfied, and Behren's method for evaluating the data was applied (8).

The LD_{50} was found by plotting a curve and by interpolation to be 0.229 cc./20 Gm. mouse. This was calculated to be equivalent to a dose of 1.15 cc./-Kg. of the alcoholic extract, each cc. of which represents 1 Gm. of the crude drug. Death occurred in from one-half to three minutes in all the animals which received lethal doses of the extract. Toxic symptoms were: cyanosis, stiffness of tail, irregular, asymmetrical and clonic convulsions, exophthalmos, micturition, and quickening, followed by slowing of respiration. Death occurred from respiratory failure, the heart continuing to beat for some time after cessation of respiration. At the end of one week all the surviving animals appeared completely normal. Both groups which served as controls responded quite passively to the 0.5% solution of NaCMC alone.

Chronic Toxicity.—Female, Sherman-Wistar rats were utilized in the chronic, oral toxicity study of the alcoholic extract *L*-8808 (Dehra Dun). One group of twelve rats received oral doses of a 1:5 dilution of the extract in a 0.5% solution of NaCMC six days weekly for a period of six weeks. These doses were equivalent to 0.5 cc. of the alcoholic extract/-Kg. rat (1.0 cc. of the alcoholic extract represented 1 Gm. of crude drug). A control group of 12 rats received equivalent volumes of the NaCMC solution alone. During the six-week dosage period, all the animals appeared entirely normal. They were then mated with healthy, Sherman-Wistar males. The young were weaned after twenty-one days, whereupon two groups of 12 male rats were selected, one group from the drugged and the other from the control litters. These weanlings were then given the drug and control injections, respectively, as outlined above. After six weeks the animals were decapitated. Gross pathological examination of the major organs and systems were negative with the exception of a large, green-colored cyst on the lungs of one of the drugged rats. It is believed that this was caused by an accidental injection injury rather than by the drug directly. All four groups of rats were weighed every third day and there was no significant gain or loss of weight in the drugged animals over that of the controls. There was also no evidence of diarrhea, gastrointestinal upset, or sedation, or hypnosis in any of the rats.

[1] "Cellulose Gum," CMC-70-M, Hercules Powder Co., Wilmington, Del.

DISCUSSION AND CONCLUSIONS

Although the experimental evidence obtained from each of the five drug varieties tested was by no means equivalent or sufficient for a quantitative comparison of their potency, a careful examination of the results discloses that there exists a difference in antihypertensive activity among different varieties of *Rauwolfia serpentina* as well as among different samples of the same variety. Of the nine samples tested, the *K* (Dehra Dun), *L*-8808 (Dehra Dun) and *P* (Himalayan) were about equally the most potent; *RKP*-207-222 and *BKC*-3490, both of which were Malabar material, were the least potent; and the *L*-7784 (Himalayan), *BK*-3690 (Himalayan), *B* (Bihar), and *BK*-3707 (Coastal Plain) were about equal in activity and approximately one-half as potent as the three most active samples. The above variations in potency together with the oftentimes wide variation in the response among different animals are exceedingly important in that they reveal the need for a reliable method of assay for purposes of standardization of the activity, both where the crude drug and the alkaloids are concerned.

The inconsistent response of the dogs to oral administration of *Rauwolfia serpentina* is difficult to understand. Following the oral doses which did cause a response, the results were excellent and indicative of an absorption procedure. Further experiments with the use of pure preparations are now in progress in an effort to settle this issue.

The results shown in Table III along with evidence not included in the table indicate that the vagi of the experimental animal may be sectioned without altering the hypotensive action or activity of the drug. Consequently, a smoother, more uniform blood pressure tracing, free from respiratory vagal effects, may be attained for assay or other comparative work. This, of course, pertains only to the alcoholic extract of the drug and may or may not apply to other preparations of *Rauwolfia*. However, the literature does cite on several occasions that the ajmaline alkaloid does not lower the blood pressure or depress the heart by acting through the vagi (9, 10).

The data of the acute and chronic toxicity studies suggest that *Rauwolfia serpentina* possesses a rather high therapeutic index and is relatively free from side effects. This coupled with its prolonged hypotensive action gives it two distinct advantages over many of the other antihypertensive agents now in use.

Exclusive of the alcoholic extracts, the pure serpentine alkaloid and the serpentine group of alkaloids were the major pressure-reducing fractions tested thus far. However, based on experiments presently being conducted but as yet incomplete, the alkaloids as separated by the *British Pharmaceutical Codex* (1949) method do not represent the total activity of the alcoholic extracts from which they were separated. Furthermore, the alcoholic ex-

tract does not represent the total activity of the crude drug. The results of additional experiments along this line, some site of action studies, and some comparative potency tests involving the recently isolated alkaloid reserpine (11–13) will be reported later.

SUMMARY

1. The antihypertensive activity of the alcoholic extracts of nine drug samples from five different varieties of *Rauwolfia serpentina* was determined. A difference in potency was found to exist among some of the varieties and among different samples of the same variety.

2. Of the alkaloidal preparations tested, serpentine and the serpentine alkaloidal group were the major blood pressure-reducing fractions.

3. Sectioning of the vagi does not alter the typical antihypertensive effect of the alcoholic extract.

4. With the exception of several adulterated samples, defecation was the only untoward side effect produced by the drug, and toxicity studies indicate a high therapeutic index.

5. The experimental evidence suggests that *Rauwolfia serpentina* is worthy of further chemical and pharmacological study.

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Book Notices

A History of the College of Pharmacy, Columbia University. By CHARLES W. BALLARD. Columbia University Press, New York, 1954. 89 pp. Illustr. 17.5 x 26 cm. Price $2.

The author of this book has been associated with the College of Pharmacy of Columbia University for 47 years, first as a student, then as a professor, and finally as Dean. He is, therefore, well qualified to write a history of the College of Pharmacy.

Established in 1829, the College of Pharmacy of Columbia University was the first in New York State. Through the years it has exerted considerable influence on legislation in the interest of public welfare such as the law that requires college graduation for licensure to practice pharmacy. Its influence was also of considerable importance in the founding of the AMERICAN PHARMACEUTICAL ASSOCIATION, the New York State Pharmaceutical Association, and the Conference of Pharmaceutical Faculties (now the American Association of Colleges of Pharmacy).

In his book, Dr. Ballard traces the role the College has played, clarifies its changes in organization, and portrays the curriculum which has developed from an essentially apprenticeship system to a standard university course of study. His presentation is of significance not only to graduates of the school, but to others interested in the development of pharmaceutical education in the United States.

Actualités Pharmacologiques. RENÉ HAZARD, Editor. Series No. 6. Masson et Cie, Paris, 1953, 224 pp. Illustr. 16.5 x 25 cm. Paper bound, uncut. Price *Fr.* 1,480.

Like its predecessors, this annual for 1953 presents reviews on a selected number of topics of timely interest. It includes papers on cardiac antifibrillants, on structural relations between sympathomimetics and sympatholytics, on pharmacological uses of tracer isotopes, on the physicochemical analysis of local anesthetics, on the active molecule in pharmaceutical synthesis, on the pharmacodynamic reaction of ganglia, on the properties of thyroid hormone, and finally on the mechanism of action alkaloids and ergot. All these reviews are competently written, and the only drawback of the book is the difficulty of finding the valuable material contained therein when needed. It apparently is not covered by any abstract service in the United States.

Pratique de l'Hibernothérapie en Chirurgie et en Médécine. By H. LABORIT and P. HUGUENARD. Masson et Cie, Paris, 1954. 256 pp. Illustr. 17 x 25 cm. Paper bound, uncut. Price *Fr.* 1,400.

Artificial hibernation is a new therapeutic approach to certain pathological conditions, particularly to traumatic and surgical shock, to some toxic

states and infections, to essential malignant hypertension, and to psychoses. It is of special interest to our profession because it is a pharmacodynamic method. It involves a "lytic cocktail," used as an autonomic-blocking agent which brings about a homeostatic neuroplegia leading to a slow-down of all biological processes (hypometabolism) which, in turn, induces hypothermia. This method has largely been developed in France by the authors of the book under discussion; it represents, therefore, an authoritative account of the experience gained. As the title indicates, the authors have pursued practical aims; they have gathered in their work the clinical accounts of numerous specialists who have adapted the general principles of hibernotherapy to their own fields of specialization. Besides reports of successful application, we also find discussions dealing with difficulties and failures.

Considering the short period of clinical experience with the method—some three years—the book can hardly be expected to be the "last word" on its subject—if there is such a thing in medicine, but it is extremely valuable because it presents a new therapy which has been little explored in this country. An English translation would probably be most welcome.

Laboratory Instruments—Their Design and Application. By A. ELLIOTT and J. HOME DICKSON. Chemical Publishing Co., New York, 1953. 414 pp. Illustr. 15 x 22 cm. Price $7.50.

The authors of this book have planned it mainly for research workers who may be called upon to devise and construct their own optical instruments. The first several chapters discuss the properties of various materials and their treatment and use in the construction of instruments. Directions are given for the preparation of drawings for workshop use and their reproduction. Other chapters are devoted to methods of construction to suit special requirements and in particular the kinematic design of instruments is discussed quite fully. In addition, vibration, insulation, sensitivity, and methods of measurement are treated. There are also chapters on glass and instructions for its use and how it may be worked to fit the needs of a number of optical instruments and devices. The book is replete with tables and diagrams which illustrate the use of lenses, mirrors, and prisms. The final chapter discusses the applications of photography in research, and is followed by several appendixes containing miscellaneous information. The book is documented to a limited extent and includes an adequate index.

A French-English Dictionary for Chemists. 2nd ed. By AUSTIN M. PATTERSON. John Wiley & Sons, New York, 1954. 476 pp. 14 x 18.5 cm. Price $6.50.

The first edition of this excellent dictionary was published in 1921. Through the years it has served as an aid to countless numbers of chemists, and also others in related fields of research, in reading and understanding articles written in the French language. In the meantime, however, many terms and meanings of words used in chemistry and related sciences have been added, and Dr. Patterson has found it necessary to increase the number of entries from 35,000 to an estimated 42,000. The increase would have been greater if many terms having identical spellings and meanings in French and in English had not been omitted in order to save space. Other space-saving devices have also been used. Despite the saving in space thus accomplished, the number of pages had to be increased from 384 in the first edition, to 476 in the second. Chemical, pharmaceutical, and related nomenclature has been revised where changes have occurred. Obsolete pharmaceutical names have, in most cases, been retained but are usually marked *Old Pharm.* or *Obs.*

Patterson's French-English Dictionary for Chemists is more comprehensive than the title indicates. It includes a general vocabulary quite beyond that one might be led to expect from the title. It is so complete in this respect that it is seldom, if ever, necessary to consult a more general type dictionary when reading French scientific literature. The term *for chemists* in the title has been broadly interpreted, and there are so many entries on technical words used in the cognate sciences that the dictionary can and does serve research workers in a most competent manner in areas other than chemistry. Terms not strictly chemical in nature are labeled freely with abbreviations such as *Biol.*, *Bot.*, *Geol.*, *Med.*, *Pharm.*, and *Physiol.*

In general, the format and style of the second edition of Patterson are the same as those employed in the first. There was no reason to introduce changes, because the original has proved so eminently successful. As in the first edition, a large number of phrases have been included, and in the words of the author, "Irregular verb forms. . .are given where they are most needed, i. e., in the vocabulary under their own spellings, and not safely concealed under the parent verb."

It is difficult for one who has used Patterson's *French-English Dictionary for Chemists*, and its companion volume, *German-English Dictionary for Chemists*, for more than a quarter of a century to be objective and not become lyrical in writing about either volume. During many years of constant use of Dr. Patterson's dictionaries this reviewer has never been disappointed. Many other users have expressed the same feeling. No other dictionary, with the possible exception of a dictionary of the English language, is capable of paying greater dividends to the chemist in the form of satisfaction than the dictionaries compiled by Dr. Patterson. He is to be congratulated upon his accomplishments in this important area.

A Textbook of Forensic Pharmacy. 3rd ed. By THOMAS DEWAR. Edward Arnold Publishers, Ltd., London, 1954. (St. Martin's Press, New York.) 287 pp. 15 x 23 cm. Price $4.

This text on forensic pharmacy is important as a clearly written exhaustive discussion of the legal climate of pharmacy in the United Kingdom. The book gains added importance because, to our knowledge, no American compilation covers this field and it may well serve as a pattern to somebody who may want to fill this gap in our pharmaceutical literature. Dr. Dewar divides his subject into three parts: Part I deals with pharmacy, poisons, and dangerous drugs; Part II with medicines; and Part III with enactments, such as the Shops Acts,

which chiefly concern retail business. There are appendices containing the Poisons List, Schedules to the Poisons Rules, a List of Poisons monographed in the *British Pharmacopoeia* and finally, a set of review questions. It is interesting to note that the book begins with a detailed account of the Pharmaceutical Society of Great Britain and the organization and functions of its Statutory Committee which exercises mainly disciplinary powers. Of equal interest is a lucid explanation of the legal implications of the National Health Service. All in all, this is a stimulating book which should certainly be in every college library.

Arzneimittel-Synthese. By H. P. KAUFMANN. Springer Verlag, Berlin, 1953. 834 pp. Illustr. 17.5 x 25.5 cm. Price *DM* 87.

We all are aware of the importance of the subject of Dr. Kaufmann's book as, with the exception of a few alkaloids, vitamins, and hormones, practically all modern pharmaceuticals are of synthetic origin. The author has covered this wide field more thoroughly and in greater detail than his avowed purpose of writing a textbook for pharmacy students would lead one to expect. It can therefore also be used advantageously by the full-fledged chemist, pharmacist, and physician. The literature, including patents, has been taken into consideration up to 1952; frequently, the author reports his own investigations and results which further enhance the value of the work. The arrangement of the material is based on the therapeutic indications while the subdivisions are grouped according to chemical constitution. This compromise does not complicate matters for the user because there is a satisfactory subject index. The book shows promise of becoming the German standard work in its field.

The Microtomist's Formulary and Guide. By PETER GRAY. The Blakiston Co., New York, 1954. 794 pp. 16 x 23.5 cm. Price $10.50.

A comprehensive compilation of methods for preparing microscope slides from biological specimens is given in Part I of the book. Each chapter deals with a specific type of slide, with a discussion of general principles and problems followed by one or more examples given in detail. Part II of the book is devoted to specific types of formulas and techniques by which they are used. A decimal numbering system necessitates much page turning, but it saves space in repeating the many formulas and stains which are given with literature references. A 114-page index containing many cross-references is a useful addition to the text material.

The book should be made available to students and to those who utilize microscope slide techniques in investigations and clinical testing procedures.

Toxicity of Industrial Organic Solvents. (Revised American Edition). By ETHEL BROWNING. Chemical Publishing Co., New York, 1953. 411 pp. 16.5 x 24 cm. Price $8.

This newly revised and enlarged edition should contribute to industrial safety and serve as an aid in the diagnosis and treatment of acute and chronic poisoning of workers in industrial plants using organic solvents in their manufacturing processes. The book presents a comprehensive summary of existing available information on the effects of a great variety of industrial solvents on animals and man. The information on which the report is based has been obtained from widely scattered sources and it is designed with the object of avoiding poisoning from the use of solvents used in industry.

The book is conveniently divided into ten chapters covering hydrocarbons; chlorinated hydrocarbons; alcohols; ethers; esters; ketones; glycols and their derivatives; amines and coal tar bases; nitro-compounds; and miscellaneous compounds.

Adressbuch Deutscher Chemiker, 1953–54. (Who's Who in German Chemistry.) Published under the auspices of the Association of German Chemists Verlag Chemie, G. m. b. H., Weinheim/Bergstrasse, Germany, 1953. 448 pp. 16 x 22 cm. Price: paper bound, *DM* 11.00; linen bound, *DM* 12.80.

This directory is indispensable for those who want either general or basic biographical information concerning German chemists residing within the frontiers of the Federated German Republic and West Berlin. It is arranged according to names and also according to places where chemists are located, thus showing concentration of the chemical industry in a few areas of Western Germany. There is also valuable information concerning the Gesellschaft Deutscher Chemiker and there are lists of the recipients of the various important chemical prizes awarded in Germany.

The book seems to be carefully edited; the format is practical.

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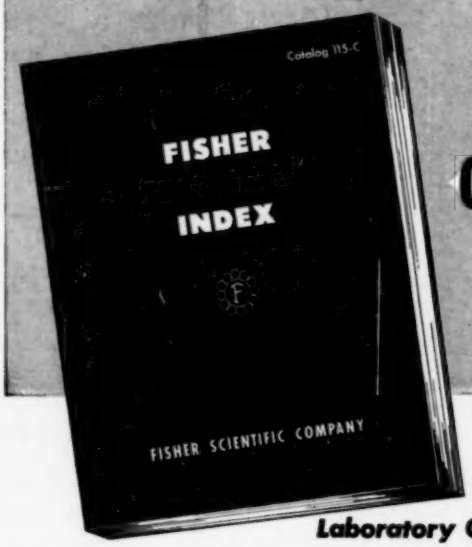

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